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THE SEPARATION OF ISOLINOLEIC ACID FROM HYDROGENATED LINSEED OIL BY CHROMATOGRAPHIC METHODS¹

BY H. W. LEMON

Abstract

When linseed oil or other oils containing linolenic acid are hydrogenated, an isomeric linoleic acid (isolinoic acid) is formed. Its concentration increases to a maximum, then decreases as hydrogenation proceeds. In view of the possible relationship of this acid to "flavor reversion", its separation in pure form has been investigated. Concentrates of isolinoic acid or its methyl ester were obtained from partially hydrogenated linseed oil by crystallizing a large proportion of the more saturated acids or methyl esters from a solvent at low temperatures. Further fractionation of such concentrates by chromatographic methods was investigated. Silica gel was found to be better for the purpose than either activated alumina or activated carbon. Methyl isolinoate was adsorbed more firmly on the silica gel than the less unsaturated esters, which were eluted by means of large volumes of hexane. Subsequent elution with chloroform removed the methyl isolinoate.

Introduction

When linseed oil or other oils containing linolenic acid are hydrogenated, an isomeric linoleic acid is formed in which the double bonds are in such positions that diene conjugation is not produced by high-temperature saponification (1, 15). In a typical hydrogenation of linseed oil, the concentration of isolinoic acid reached a maximum of 18% of the total fatty acids at an iodine value of approximately 100, then decreased on further hydrogenation.

Evidence has been presented that decomposition of isolinoic acid may give rise to the characteristic odor and flavor that develops in partially hydrogenated linseed oil, particularly on heating (15, 16). This deterioration is commonly called "flavor reversion".

In view of the possible relationship of isolinoic acid to flavor reversion, the separation of the acid in pure form by (a) fractional crystallization from a solvent at low temperature and (b) chromatographic methods has been investigated. The first method has been applied successfully by Brown *et al.* to the separation of a number of pure fatty acids (3, 4, 10, 11). The second method has been investigated by Cassidy (5, 6, 7), Kaufmann (13, 14), Graff and Skau (12), Dutton (9), Swift, Rose, and Jamieson (19), Riemenschneider, Herb, and Nichols (18), Claesson (8), and others. It was previously reported

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(15) that an isolinoic acid concentrate of iodine value 152 was separated by the crystallization procedure of Brown *et al.*, but attempts to separate the pure acid by this method were not successful. Therefore further purification of the concentrates by chromatographic methods has been tried, and this work will be described in this paper.

Experimental

Linseed oil hydrogenated to an iodine value between 75 and 80, and containing no trace of linolenic acid, as indicated by the analytical method of Mitchell, Kraybill, and Zscheile (17), was saponified and the mixed fatty acids were separated. A 5% solution of the mixed acids in acetone was cooled to -20°C . while being agitated; this caused considerable crystallization. Filtration was achieved by suction, using a porous stone immersed in the mixture. The crystals were washed with a little acetone at -20°C ., the mixture filtered, and the filtrates combined. This solution was treated in the same manner, but at -50°C ., and the resulting filtrate was also treated in the same manner, but at -65°C . The filtrate was now concentrated to about one-quarter of its volume and again allowed to crystallize at -65°C . Acetone was removed from the final filtrate by distillation, and the resulting isolinoic acid concentrate had an iodine value of 150 to 155. Attempts at further purification by crystallization were not successful. Some of the isolinoic acid concentrate prepared in this way was converted to the methyl esters and distilled *in vacuo*.

A concentrate of methyl esters that proved to be a satisfactory starting material for chromatographic separations was obtained more simply, in the following manner. The hydrogenated linseed oil was converted to methyl esters by alcoholysis, using the method of Wright, *et al.* (20). A solution of 100 gm. of esters in 400 ml. of hexane was stored in a cabinet at -18°C . for 18 to 24 hr. The mass of crystalline esters was separated by filtration, the filtrate concentrated, chilled to -70°C ., and again filtered. Esters with an iodine value of about 120 were obtained.

Activated alumina, activated carbon, and silica gel were used as adsorbents. The alumina samples used were chromatographic grades with an activity equivalent to 1 or 2 on the Brockmann scale (2). Samples of activity 4 and 5 on this scale were prepared from these by exposure to moist air.

The activated carbon used was Darco G 60, manufactured by Darco Corporation, New York. It was mixed with an equal weight of Johns-Manville Hyflo Super-cel.

The silica gel was a commercial grade, 28-200 mesh, obtained from the Davison Chemical Corporation, Baltimore, Maryland.

The adsorbents were packed in glass columns and were flooded with the solvent to be used. The solution of fatty acids or esters was then added to the top of the column. Large volumes of solvent were allowed to pass through the adsorbent, the eluate was collected in fractions, the solvent was

removed by distillation, the residue weighed, and refractive index and iodine number determinations made when possible.

Results

(a) Activated Alumina

A number of experiments were tried using activated alumina of various activities, and various ratios of methyl esters to alumina. The solvent was petroleum ether. Methyl isolinoleate adhered most firmly to the alumina, and was difficult to remove. It is probable that a method could be developed with the use of this adsorbent, but, owing to the difficulties encountered, it was abandoned.

(b) Activated Carbon

Fractionation of both fatty acid and methyl ester concentrates by means of activated carbon was investigated. In both cases, a fraction with a higher iodine value than that of the original concentrate came through the column at the beginning of elution with petroleum ether, followed later by more saturated fractions. The results of one experiment using a concentrate of methyl esters are given in Table I. The process was very slow, and pure isolinoleic acid was not obtained by one passage of the acids or esters through the column.

TABLE I

SEPARATION OF METHYL ESTERS CONTAINING METHYL ISOLINOLEATE, BY ADSORPTION ON ACTIVATED CARBON

Contents of column:— 15 gm. Darco G. 60 } mixed
15 gm. Filter aid }
1 gm. of methyl esters, iodine value 141

Solvent:— Petroleum ether

Sample No.	Volume of eluate, cc.	Weight of esters, gm.	Iodine value of esters
1 to 2	125	0.02	
3 to 6	100	0.21	162
7 to 10	100	0.22	151
11 to 15	125	0.12	128
16 to 26	275	0.16	

NOTE:— Esters recovered 0.73 gm.

(c) Silica gel

Silica gel proved to be the most practical of the three adsorbents. When petroleum ether or hexane was used as solvent, methyl isolinoleate in a methyl ester concentrate was more firmly adsorbed than the more saturated esters, which were separated from the silica gel by elution with large volumes of the solvent. The adsorbed methyl isolinoleate was released by passing chloroform through the column, and the esters obtained had an iodine value close to the theoretical value of 172.3 for the pure ester. The ratio of esters to adsorbent was 1 to 10. The results of three experiments are given in Table II.

TABLE II

SEPARATION OF METHYL ISOLINOLEATE BY ADSORPTION ON SILICA GEL

Volume of eluate, cc.	Weight of esters eluted, gm.	Refractive index, 20° C.	Iodine value
<i>Experiment I. 20 gm. silica gel. 2.1 gm. methyl esters (iodine value, 144). Solvent: petroleum ether, followed by chloroform.</i>			
Petroleum ether			
700	0.016	1.4566	
500	0.119	1.4530	
500	0.118	1.4539	
500	0.179	1.4545	
500	0.178	1.4549	
500	0.166	1.4560	
500	0.159	1.4570	
1000	0.162	1.4601	
1200	0.143	1.4608	
Total	5900	1.240	
Chloroform			
100	0.703	1.4615	173
100	0.038	1.4650	
100	0.049	1.4670	
Total	300	0.790	
<i>Experiment II. 250 gm. silica gel. 25 gm. methyl esters (iodine value, 145). Solvent: petroleum ether, followed by chloroform</i>			
Petroleum ether			
65000	10.5	1.4546	103
Chloroform			
500	6.1	1.4606	167
500	3.4	1.4613	170
500	0.95	1.4616	174
500	0.46	1.4636	181
<i>Experiment III. 250 gm. silica gel. 25 gm. methyl esters (iodine value, 117). Solvent: hexane, followed by chloroform</i>			
Hexane			
73000	16.1	1.4551	96
Chloroform			
500	3.2	1.4598	168
500	1.8	1.4606	170
500	1.0	1.4608	172
1500	0.8	1.4633	173
1000	0.3	1.4648	173

The steadily rising refractive indices of the fractions separated from the adsorbent in Expt. I by elution with petroleum ether show that the most saturated esters came through the column first, followed by fractions of increasing unsaturation, undoubtedly containing some quantity of methyl isolinoleate. It is important that elution with hexane or petroleum ether be continued no longer than is necessary to remove the more saturated esters if a good yield of methyl isolinoleate is to be obtained.

On eluting the column with chloroform, fractions varying in iodine number between 167 and 180 have been obtained. As the theoretical iodine number for linoleic acid is 172.3, it is obvious that there was a small amount of a more unsaturated substance present. The refractive index of the best methyl isolinoleate samples was approximately 1.4613 at 20° C. When elution with chloroform was continued, more viscous fractions with higher refractive index values were obtained. These may have been polymers.

The linolenic and linoleic acid content of the methyl isolinoleate concentrate, of the purified methyl isolinoleate from the adsorption column, and of the esters eluted from the column with hexane have been estimated by the spectral method (17). The amounts of these acids present were so small that accurate values were not obtained. In each case linolenic acid was of the order of 0.1% and linoleic acid about 1.0% of the total fatty acids.

The ultraviolet absorption of a solution of the purified methyl isolinoleate in iso-octane has been determined. This revealed that it contained a small amount of a substance having triene conjugation. The extinction value ($E_{1\text{cm}}^{1\%}$) at 268 m μ was 1.4.

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THE SIGNIFICANCE OF AN ABSORPTION BAND AT 968 CM.⁻¹ IN THE INFRARED SPECTRUM OF METHYL ISOLINOLEATE¹

BY H. W. LEMON AND C. K. CROSS

Abstract

The infrared absorption spectrum of methyl isolinoleate, separated from the methyl esters of hydrogenated linseed oil fatty acids, has a well defined absorption band with maximum absorption at about 968 cm.⁻¹ As an identical band was found in the spectra of fatty acids or esters after isomerization with selenium, it is attributed to the presence of double bonds with a *trans*-configuration. It was found that the same band was present in the spectra of samples taken during hydrogenation of oils, and that its intensity increased to a maximum, then decreased as hydrogenation proceeded. It is concluded that hydrogenation is accompanied by a *cis*-to-*trans* change in some of the double bonds of the fatty acids, and that methyl isolinoleate has at least one double bond with a *trans*-configuration.

Introduction

In the course of an investigation of the hydrogenation of linseed oil, it was found that an isomeric linoleic acid was produced by hydrogenation of linolenic acid (4). Some evidence was presented that indicated that decomposition of isolinoleic acid, as it was called, may be at least partly responsible for the characteristic flavor deterioration of shortening made from hydrogenated linseed oil (4, 5). A method of separating isolinoleic acid in reasonably pure form has also been described (6).

In this paper, the infrared absorption spectra of methyl isolinoleate, of methyl linoleate, of samples taken during the hydrogenation of various vegetable oils, and of oils or esters that have been heated to 200° C. in the presence of selenium will be compared, with reference to an absorption band in the region of 968 cm.⁻¹ that was found in the spectrum of methyl isolinoleate. The significance of this band will be discussed.

Experimental

Methyl isolinoleate was separated from the methyl esters of hydrogenated linseed oil fatty acids by adsorption on silica gel (6). Methyl linoleate was prepared by the method of Rollett (11), and a methyl linoleate concentrate of iodine value 155 was prepared from the fatty acids of sunflower seed oil, after removal of as much as possible of the more saturated acids by crystallization from an acetone solution at -60° C.

In selenium isomerizations, 0.3% selenium powder was used. The oils were heated for three to four hours at 220° C. and were agitated by means of a current of carbon dioxide through the oil.

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Hydrogenations were conducted in a steel pressure vessel already described (4). The oils were alkali refined. A commercial nickel catalyst was used, obtained from the Rufert Chemical Company, Seymour, Connecticut. The catalyst concentration was 0.4% of the weight of the oil. The temperature of hydrogenation was 140° to 150° C., and electrolytic hydrogen at 20 lb. gauge pressure was used. Samples were taken periodically from each hydrogenation for iodine value determinations and infrared absorption measurements.

Iodine values of the samples were determined by the official method of the American Oil Chemists' Society (2).

Some of the samples were separated into solid and liquid acids by the modified Twitchell method of the American Oil Chemists' Society (2). The liquid acids were separated from the filtrates. Both solid and liquid acids were converted to the methyl esters, as the free acids have an absorption band at about 940 cm^{-1} that interferes with measurement of the absorption at the peak of the 968 cm^{-1} band when it is of low intensity.

The infrared absorption curves were obtained with the use of a Perkin-Elmer recording spectrometer. The hydrogenated samples and the methyl esters of the solid and liquid fatty acids were dissolved in carbon disulphide to a concentration of 10%, and cells of 0.1 mm. thickness were used. No solvent was used with the methyl isolinoleate and methyl linoleate samples, and the cell thickness was 0.025 mm.

Results

This chief difference between the infrared absorption spectrum of methyl isolinoleate and that of the two methyl linoleate samples is shown in Fig. 1. The methyl isolinoleate spectrum had a well defined absorption band with maximum at 968 cm^{-1} which was absent in the spectrum of the methyl linoleate concentrate prepared from sunflower seed oil, and was very weak in that of the methyl linoleate prepared by the method of Rollett. No such band was found in the spectra of any of the unhydrogenated vegetable oils examined, but it was present in the spectra of partially hydrogenated samples and in the spectra of oils or esters after heating at 200° C. in the presence of 0.3% selenium for three to four hours.

In Fig. 2 the per cent absorption at the peak of the 968 cm^{-1} band for each hydrogenated sample as compared with the absorption of the unhydrogenated oils at the same frequency is plotted against the iodine value of the sample. In every case the absorption intensity increased to a maximum, then decreased as hydrogenation proceeded. The iodine values at the point of maximum absorption were different for each oil, and were almost directly related to the iodine value of the unhydrogenated oil. The maximum absorption value that developed on hydrogenation was also related to the degree of unsaturation of the unhydrogenated oil, and was considerably greater for linseed and soybean

oils than for those that did not contain linolenic acid. For linseed oil, the iodine value at the point of maximum absorption was approximately the same as that reported previously at which oleic and isolinoleic acids were at a maximum (4).

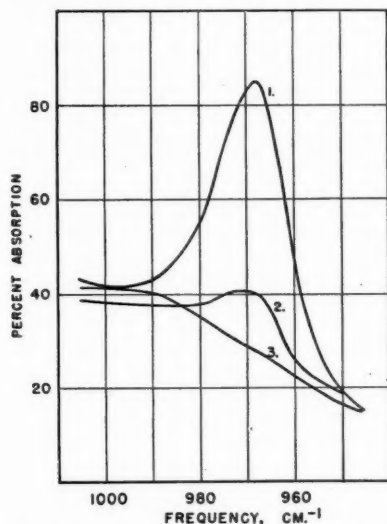


FIG. 1. A portion of the infrared absorption spectra of: 1, Methyl isolinoleate; 2, methyl linoleate prepared by method of Rollett; 3, methyl linoleate concentrate prepared from sunflower seed oil.

The 968 cm.^{-1} band was found in the spectra of the methyl esters of both the solid and liquid fatty acid fractions of hydrogenated peanut oil samples. The per cent absorption values at the peak of the band are given in Table I.

TABLE I

ABSORPTION INTENSITY AT 968 cm.^{-1} FOR METHYL ESTERS OF SOLID AND LIQUID FRACTIONS OF HYDROGENATED PEANUT OIL SAMPLES

Iodine value of sample	Per cent solid acids	Iodine value solid acids	Per cent absorption, methyl esters of solid acids	Iodine value liquid acids	Per cent absorption, methyl esters of liquid acids
80	21	8	12.9	94	18.7
70	27	14	16.8	91	18.7
58	40	20	24.1	85	29.2
39	51	15	23.2	77	35.4
19	72	5	8.8	61	41.1
11	79	3	6.7	46	32.3
6	83	1	6.7	20	20.5
3	85	0.6	6.7	16	12.9

When a mixture of linseed oil and the nickel catalyst was heated at the hydrogenation temperature ($150^{\circ}\text{C}.$) in an atmosphere of nitrogen rather than hydrogen for the length of time required for a hydrogenation, the spectrum showed little trace of the 968 cm^{-1} band. However, it did appear after very prolonged heating of the oil in the presence of catalyst.

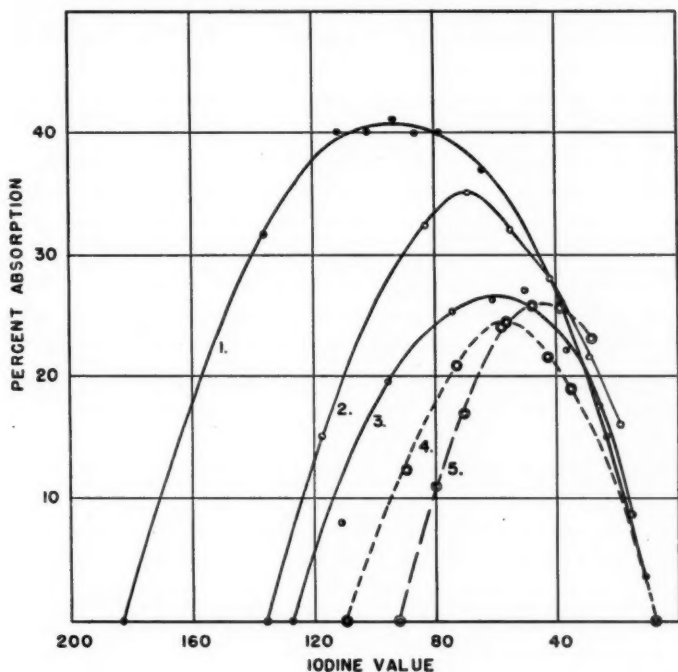


FIG. 2. Effect of hydrogenation on the relation between per cent absorption at the peak of the 968 cm^{-1} band and iodine value for: 1, linseed oil; 2, soybean oil; 3, corn oil; 4, cottonseed oil; 5, peanut oil.

Discussion

Absorption bands close to 968 cm^{-1} have been found in the spectra of certain olefins by several investigators. Thompson and Torkington (13) and Thompson and Whiffen (14) have reported that compounds of the type $\text{R}_1\text{—CH=CH—R}_2$, such as butene-2, pentene-2, and octene-2, have an intense absorption band between 960 and 970 cm^{-1} . They attribute this to deformational motions of the CH bonds contiguous to the double bond. Sheppard and Sutherland (12) have reported absorption bands in this region in the spectra of the products of reaction of certain olefins with hydrogen sulphide. Rasmussen and Brattain (10) have also reported a strong band in this region in the spectra of certain olefins. They have concluded that it is characteristic of the *trans*-form of the CHR=CHR system.

In the case of unsaturated fatty acids, the absorption band at 968 cm^{-1} is apparently also associated with a *trans*-configuration, as the same band is found in the spectra of unsaturated fatty acids, or their esters, after heating for three hours in the presence of 0.3% selenium, which is a well known method of obtaining an equilibrium between *cis*- and *trans*-isomers (3). Rao and Daubert (9) have reported that the infrared spectra of vaccenic and elaidic acids have an absorption band at this frequency, whereas that of oleic acid has not; this was taken as further evidence that vaccenic acid has a *trans*-configuration. It has been reported also that the spectrum of synthetic vaccenic acid has this absorption band (1).

It is concluded, therefore, that the isolinoleic acid produced by hydrogenation of linolenic acid has at least one double bond with a *trans*-configuration. The fact that no increase in the absorption at 968 cm^{-1} resulted when a quantity of methyl isolinoleate was heated in the presence of selenium may mean that both double bonds have a *trans*-configuration.

It is also concluded that the intensity of absorption at the peak of the 968 cm^{-1} band is a measure of the extent of the *cis*-to-*trans* change that is associated with the hydrogenation of unsaturated fatty acids. It was first reported by Moore (8) in 1919 that such a change takes place. He found that the largest component of iso-oleic acid produced on hydrogenation of ethyl oleate was elaidic acid, the *trans*-isomer of oleic acid. He pointed out that the change was an equilibrium reaction, as not only was there a formation of solid elaidic acid on hydrogenation of oleic acid, but also there was a corresponding formation of oleic acid on hydrogenation of elaidic acid.

The fact that the absorption band at 968 cm^{-1} for the liquid acids from hydrogenated peanut oil samples was somewhat more intense than that for the corresponding solid acids indicates that the iso-oleic acid precipitated by the Twitchell method (2) is not the only *trans*-acid that is formed on hydrogenation. It is probable that a considerable proportion of all of the unsaturated acids are *trans*-isomers. What the significance of this may be with regard to the stability or other properties of the products is not at once apparent. It is almost certainly the reason for some of the difficulty that is encountered when the spectral method of fatty acid analysis of Mitchell, Kraybill, and Zscheile (7) is applied to partially hydrogenated oils.

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FLOW PROPERTIES OF DISPERSIONS OF CALCIUM SOAPS IN HYDROCARBON OIL¹

G. B. MOSES AND I. E. PUDDINGTON

Abstract

The flow properties of dispersions of calcium soaps in hydrocarbon oils have been studied over a range of temperature from 35° to 90° C., and rates of shear from 500 to 16,000 sec.⁻¹ by extrusion through a capillary plastometer. The thixotropy of the system falls as the temperature is increased to 50° C. and then rises to a maximum at 65° C., after which it falls regularly. This behavior is due to the thermal weakening of the thixotropic bonds, followed by a strengthening of these bonds due to incipient swelling on the surface of the soap. As the surface of the soap particles continues to swell, cohesion of the particles increases to such an extent that breakdown does not take place and the coefficient of thixotropy falls to very small values. The coefficient of thixotropy found by this method bears a linear relation to the hardness of the dispersion as determined by a falling cone.

Introduction

Lubricating greases are normally stable dispersions of metallic soaps in hydrocarbon lubricating oils. In consistency they may vary from very soft plastic masses to moderately hard solids. Their usefulness in lubrication depends largely on their non-Newtonian flow properties, i.e., the force per unit area necessary to produce continuous flow is not proportional to the rate of shear. They belong to a class of substances which, at constant temperature, show a reversible decrease in apparent viscosity with increasing rate of shear; substances with such properties have been termed thixotropic. The thixotropic properties of greases are responsible for their ability to stay for extended periods in or near the location where lubrication is desired, since at zero rates of shear their viscosity approaches infinity and they do not flow. On the other hand, at high rates of shear the ideal grease should have a viscosity of the same order of magnitude as the oil from which it is made and cause as small a load as possible at the bearings. Greases thus should have the ability to provide good lubrication at high speeds on bearing surfaces and act as self-sealing containers near the edges of the bearings where the rate of shear is low. Oil under similar conditions would flow away from the bearings continuously. Obviously a thorough knowledge of the flow properties of lubricating greases under widely varying conditions of temperature and shear is of prime importance.

The solid phase of conventional lubricating greases is commonly composed of one of the metallic soaps; calcium, sodium, lithium, aluminum, or barium. Each grease possesses individual properties that make it advantageous for some uses but place it at a decided disadvantage for others. Greases containing calcium soaps form the major portion of those in use today, and the results presented here will be confined to that type of grease. These greases are

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unique in that small quantities of water (1 to 3%) are necessary to produce stability in the system although the final product is highly water resistant.

Previous work on the flow properties of greases has been limited to rather narrow ranges of temperature, shear, and composition. Rochner and Robinson (21) determined the apparent viscosities of calcium base greases to show that normal increases in the soap concentration do not necessarily lead to increases in apparent viscosities. Arvison (3, 4) has shown that the apparent viscosity at high shear depends mainly on the mineral oil, the difference from the viscosity of the oil being proportional to the soap content, from which he derives a "soap factor". Beerbower *et al.* (5) showed that the variation of the apparent viscosity with the rate of shear can be used to separate the effect of the soap from that of the oil, and shows each in its proper perspective. Sometimes the reverse of results indicated from cone penetration tests is found by this approach.

Viscosities have been measured (1, 7, 15, 16, 20), by penetration of a double cone, moving parallel plates, rotating cones and cylinders, and capillary type pressure viscometers. Bulkley and Bitner (8) employed a capillary tube type having a cylinder on each end of the tube permitting a series of tests on the same sample of grease by forcing the material in one direction and then reversing the flow.

Experimental

The viscometer used here was of the capillary tube type. A steel cylinder, 7 in. long, 1½ in. outside diameter, and 1 in. inside diameter, having a steel capillary tube screwed into one end and a pressure fitting at the other, was connected through copper tubing, a manifold, and a ballast tank to a nitrogen cylinder. Pressure was supplied by the nitrogen cylinder and measured with a mercury manometer and gauges mounted on the manifold.

The cylinder was filled with grease, care being taken to exclude air pockets, placed in an automatic electrically heated thermostat, consisting of a thick-walled aluminum cylinder, and heated to the required temperature. After the cylinder had been maintained at a single temperature for one hour to allow it to come to equilibrium, samples of the grease were extruded at various pressures, the actual pressure depending on the temperature and soap content of the grease. From the time of extrusion and the weight of the grease extruded, the apparent viscosity and rate of shear were calculated.

The apparent viscosity was calculated from Poiseuille's formula

$$\eta = \frac{\pi r^4 p l}{8 v l}$$

and the value for the reciprocal of the rate of shear from the equation

$$\frac{1}{\sigma} = \frac{\pi r^3 t}{4 v}$$

where all the symbols have their usual meaning.

The greases used were made up from a No. 3 cup grease containing 16.5% of calcium soap and a No. 5 cup grease containing 31.1% of calcium soap, by diluting them with the same oil used in their manufacture. The soap contents were found by ashing and by extraction with chloroform, while the densities of the two basic greases were determined with a dilatometer. Values for the diluted samples were calculated from the known densities of the oil and the concentrated sample. The oil used had the following inspections: viscosity at 100° F., 308 S.U.S.; viscosity index, 74. Temperatures higher than 90° C. were not used because the grease began to separate into two phases and reproducible values for the amount extruded could not be obtained.

The two basic greases used in the work were representative of standard commercial products, the soaps having been prepared from natural glycerides. These were supplied gratuitously by Imperial Oil Ltd., to whom acknowledgment is made.

The radius of the capillary was found to be 0.0368 cm. by weighing the thread of mercury required to fill it, while a length of 2.6111 cm. was obtained using a micrometer caliper. This gives a length-to-diameter ratio of 36 : 1.

The use of a capillary in examining the viscous properties of non-Newtonian materials is often criticized on the grounds that the conditions of shear are not definitely known. However, any device involving concentric cylinders or cones where conditions of shear are known is troublesome to control thermostatically over a wide temperature range, and for this reason capillaries were used in this work. This step appears to have been justified, since values obtained for the apparent viscosity at different rates of shear were essentially the same when the length and radius of the capillary were varied considerably from the one actually used in the major part of this work. Thixotropic equilibrium appears to have been established almost instantaneously at the rates of shear encountered in the capillary.

Results and Discussion

The reproducibility of the experimental results was good. Any inconsistencies could be traced to air pockets or lack of homogeneity in the grease, and these results were discarded. Typical rate of shear vs. shearing stress curves for a range of soap concentrations are shown graphically in Fig. 1.

It is obvious from Fig. 1 that the viscosity of the greases is a function of the rate of shear, and their behavior according to the generally accepted definition, may be termed thixotropic. Thixotropy in systems where the particles of the solid phase are microscopic in size is due to a spontaneous agglomeration of these particles when the system is at rest and a deflocculation of the agglomerates when the system is sheared (2). The addition of small quantities of a third phase that preferentially wets the solid particles has a remarkable effect on the agglomeration. It has been shown (12) by Gally and Puddington that additions of water greatly increase the equilibrium sedimentation volume and greatly reduce the sedimentation time, for suspensions of a number of solids having hydrophilic surfaces, suspended in

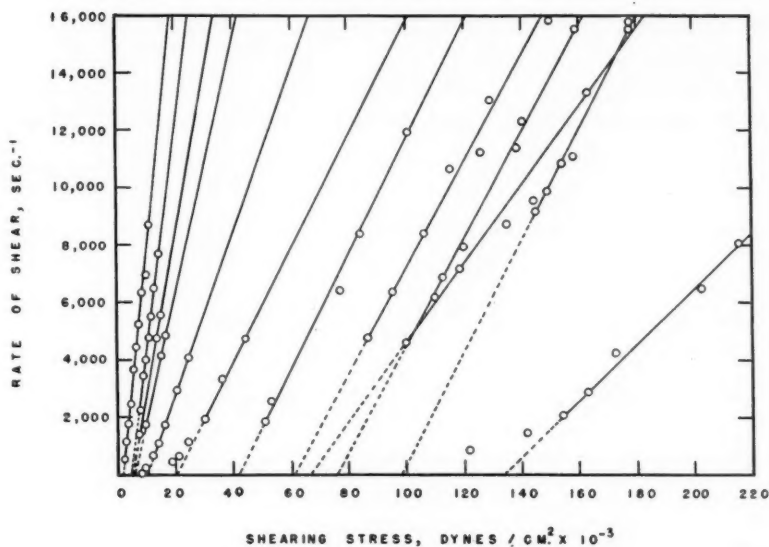


FIG. 1. Change of rate of shear with shearing stress. From right to left, consecutive graphs represent decreasing soap concentration.

organic media. These changes are accompanied by marked viscosity changes. Kruyt and van Selms (17) have shown that the same behavior is observed if small quantities of hydrocarbons are added to finely divided coal suspended in water. It is also probable that particle shape plays an important part in this phenomenon.

Goodeve (14) has advanced the theory, based on this conception of thixotropy, that under conditions of uniform shear, an equilibrium should be established where the rate of deflocculation due to shear should be equal to the rate of spontaneous agglomeration, and has derived the following formula relating viscosity and shear.

$$\eta - \eta_0 = \frac{\theta}{\sigma}$$

where η = the apparent viscosity of the system,

η_0 = the residual viscosity

σ = shear

θ = the coefficient of thixotropy.

Obviously if a system conforms with this equation a plot of η vs. $\frac{1}{\sigma}$ should give a straight line. This was found to be the case, at high rates of shear, for a number of systems, and the coefficient of thixotropy, θ , is defined by the limiting slope of the viscosity - reciprocal shear curve. Extrapolation of this curve to $\frac{1}{\sigma} = 0$ gives the value for the residual viscosity, i.e., the viscosity

of the suspension where no agglomerates exist and the solid phase particles are independent of one another.

The experimental results in the present work have been examined on the basis of Goodeve's theory. Typical data are presented graphically in Fig. 2,

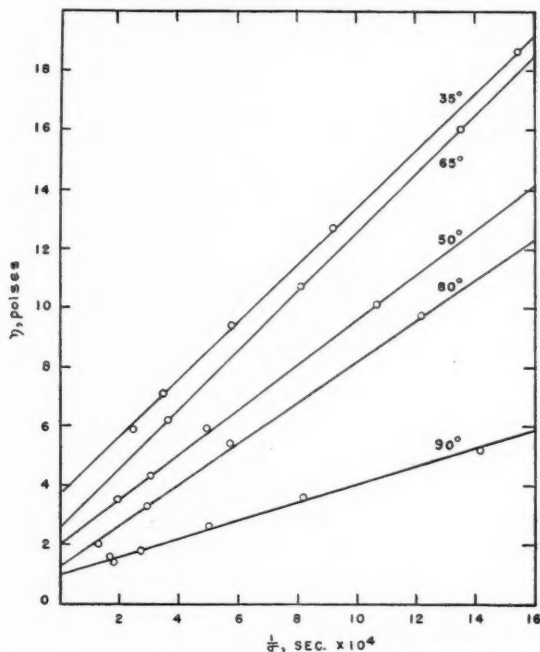


FIG. 2. Change in apparent viscosity with the reciprocal of the rate of shear for grease containing 16.5% of soap.

where the apparent viscosity in poises is plotted against the reciprocal shear (seconds) for a single soap concentration at five different temperatures. In all, the flow characteristics were measured and the data plotted in this manner for 11 different soap concentrations. The tabulated results are shown in Table I, where values of both the residual viscosity, η_0 , and the coefficient of thixotropy, θ , are recorded for the different temperatures.

Although the system was examined in detail at rates of shear up to 16,000 sec^{-1} , in a few experiments this range was extended to 220,000 sec^{-1} without any significant change in the results presented here.

The variation of the coefficient of thixotropy with temperature is shown in Fig. 3. At all concentrations below 27% of soap the values of θ fall to a minimum at 50°C. followed by a rise to a maximum at 65°C., after which the value decreases regularly and the indications are that it would reach zero in the vicinity of 100°C. Actually this point could not be checked

TABLE I

EXPERIMENTAL VALUES OF θ AND η_0 FOR DISPERSIONS OF CALCIUM SOAPS IN HYDROCARBON OILS

Temp., °C.		35°	50°	65°	80°	90°
% Soap	θ					
	η_0					
11	θ	4300	3650	2300	1800	1200
	η_0	1.28	0.57	0.44	0.33	0.24
13	θ	5600	4200	3800	2800	1550
	η_0	1.63	0.91	0.54	0.23	0.24
14	θ	6100	5300	4500	3500	2100
	η_0	2.20	1.03	1.12	0.47	0.28
16.5	θ	9700	7600	10100	7200	2800
	η_0	3.7	2.0	2.5	1.2	1.0
18	θ	22000	17900	21000	12400	4500
	η_0	4.35	2.8	2.8	1.5	1.1
22	θ	44200	32400	33600	17900	12250
	η_0	4.8	3.5	4.2	2.5	1.4
25	θ	60700	47000	50000	31000	17500
	η_0	5.5	3.0	2.1	1.85	1.65
26	θ	74200	53300	56700	33500	18600
	η_0	5.9	4.0	3.3	3.1	2.6
27	θ	82100	62300	62300	42700	28500
	η_0	6.3	3.8	3.8	3.0	1.9
29	θ	119700	77200	73400	59800	32750
	η_0	3.6	4.9	2.7	2.65	2.15
31.1%	θ	145200	135000	107000	77300	52500
	η_0	8.7	4.3	5.9	2.9	1.7

 θ , dynes per cm.² η_0 , poises.

experimentally, as the grease started to separate at temperatures above 90° C. and the viscosity readings were unreliable.

The marked decrease in θ is surprising in view of the fact that Arnold and Goodeve found an extremely small temperature coefficient with carbon black in oil. However, the temperature range here is larger and the volume concentration of solid phase much higher than those used by Goodeve. Further, this system contains water, while the carbon black in oil was essentially

anhydrous. Kruyt and van Selms (17, 18) have recently pointed out that plasticity in many suspensions containing small amounts of a third phase can be accounted for on the basis of the interfacial surface tension of the

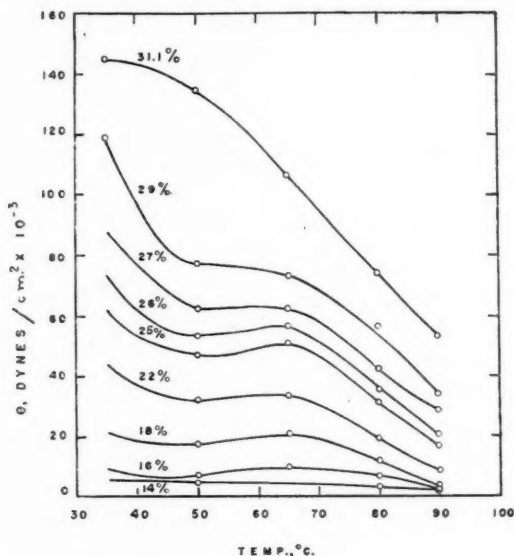


FIG. 3. Change in the coefficient of thixotropy with temperature at different soap concentrations.

third phase, which actually forms liquid bridges between the individual particles of the suspension, against the liquid medium of the suspension. An explanation of this sort would necessitate a reduction in the coefficient of thixotropy with increasing temperature, proportional to the product of the surface tension lowering and the number of bonds present. In the present instance the lowering of θ is proportional to the concentration of the solid phase (Fig. 4), a result to be expected from the above consideration.

The increase in θ following the minimum at 50°C. is probably due to incipient swelling of the surface of the soap particles, which produces considerable adhesion when the soap particles come in contact, and a change in the mechanism of thixotropy. This adhesion would be expected to increase with rising temperature to a point where the shear produced under the experimental setup would no longer be able to disintegrate the agglomerates, and the value of θ would go through a maximum and fall toward zero. Under these conditions the solid phase would form a clot and the liquid might be lost by syneresis, or the system remain in a highly swollen condition. Actually with calcium base greases of these concentrations the former usually occurs.

It is quite likely that thixotropy in these systems is a combination of the natural tendency of the soap particles to agglomerate owing to their polarity

and the bridging effect of the added water. At low temperatures the effect of the water predominates, while above 50° C. the incipient swelling of the soap causes the natural adhesion to become more pronounced. This swelling

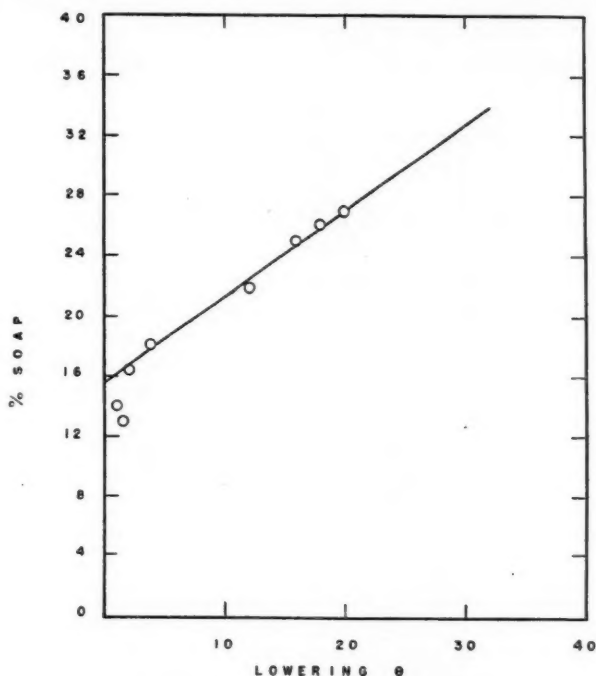


FIG. 4. Change in the lowering of the coefficient of thixotropy between 35° and 50° C. with soap concentration.

phenomenon may not attain equilibrium quickly nor is it likely to be strictly reversible, since temperature gradients are usually important in this sort of change. Obviously, it has been found of considerable practical importance, since lime base greases of this type are never recommended for use at temperatures exceeding 60° C. In support of this incipient swelling hypothesis, it was noted experimentally that a sample of soap from which most of the oil was removed became noticeably sticky at 50° to 60° C.

At high concentrations of soap (31.1%) no marked loss in the thixotropy was noted until temperatures higher than 50° C. were reached. At concentrations as high as this it is probable that disintegrated agglomerates must re-form as soon as shearing forces decrease, since the concentration is sufficiently high that each soap particle is in contact with its neighbors and the increased bond strength due to the presence of water has little effect on the size of the agglomerates formed, and hence on θ . When the temperature is

raised sufficiently to give increased adhesion the agglomerates are not broken down and the coefficient of thixotropy falls in the normal fashion.

According to Goodeve's "impulse theory" of thixotropy (2) θ should be proportional to the square of the concentration of the solid phase, and this was actually the case with carbon black suspended in oil. Fig. 5 shows a plot of $\log \theta$ against $\log C$ for the series of calcium base greases. The slope here

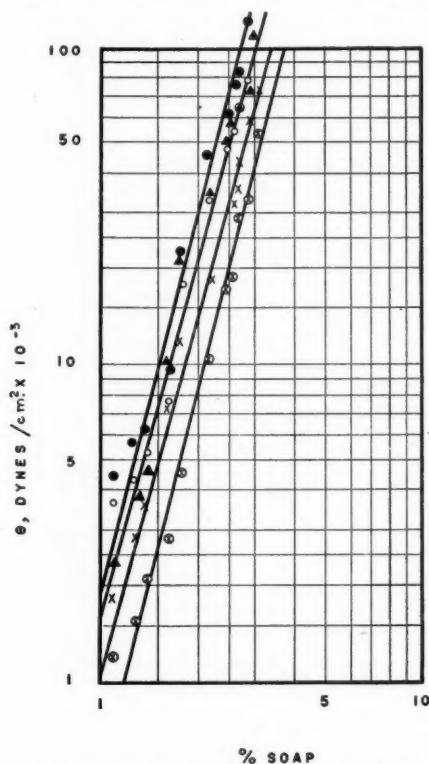


FIG. 5. Change in the coefficient of thixotropy with soap concentration. From left to right the graphs represent 35°(●), 50°(○), 65°(▲), 80°(×), and 90° C.(⊗).

is 3.7 rather than 2 predicted by the theory. This increase in θ with solid phase concentration is very probably due to the high ratio of the length to the width of the calcium soap particles compared to the substantially regular particles of carbon black. In support of this explanation, a series of sodium base greases in which the soap particles are purposely made short gave a slope of 2.8 in a similar plot. This point will be investigated further, however.

The dependence of the viscosity of a solution on the shape and size of the dispersed molecules has been the subject of numerous investigations, especially

in connection with macro-molecules of high molecular weight. Einstein's original formula for spherical solute molecules

$$\eta = \eta_0 \left(1 + \frac{5}{2} C \right),$$

where C is the volume concentration of solute and η and η_0 are the viscosities of solution and solvent respectively, has been amended as follows (19) to take account of departure from regular shape:

$$\eta = \eta_0 \left(1 + \frac{5}{2} C + \frac{f^2}{16} C \right),$$

where f is the length-to-width ratio of the rod shaped molecules.

This formula has been checked (10) by measuring the viscosities of suspensions containing very finely divided solids. It holds, however, only when the solid particles are independent of one another in the suspension and do not agglomerate. Since, at infinite rates of shear all particles in a suspension are independent and no thixotropic effects are present it should be possible to solve the above equation for f from the extrapolated values of η_0 in Fig. 2. This was done using the values of η_0 at 35° C. over the whole concentration range. The value of f obtained was 23, and this was constant for the complete concentration range. This value appears to be of the right order when one examines electron micrographs of similar greases (11) which show the actual soap particles magnified to such an extent that they can be measured readily.

The variation of η_0 with temperature and soap concentration is shown in Fig. 6. Here again the values of η_0 pass through a minimum at 50° C., followed by a maximum at 65° C. after which the values fall rapidly. The decrease in η_0 up to 50° C. is the normal lowering of the viscosity of the oil with increased temperature. The increase in the apparent viscosity is probably caused by absorption of oil by the soap during the swelling, which effectively increases the volume concentration of the solid phase and consequently the viscosity. The maximum is reached when this effect is just balanced by the formation of true solution or the soap melting. The continued fall in η_0 with increasing temperature above this maximum is normal.

HARDNESS

Cameron has recently pointed out (9) that it should be possible to measure the hardness of plastic materials if the measurement is carried out within the relaxation time of the material in order that the result will not be complicated by any flow in the sample during the test. For greases the relaxation time was calculated to be between 3 and 3×10^{-2} sec., and his measurements were carried out in times of the order of 0.8×10^{-2} sec. The method used consisted in allowing a cone of known weight to fall from a predetermined height into the grease, and expressing the hardness in gram-centimeters per cubic centimeter displaced or grams per square centimeter. The experimentally determined hardness was found to be constant over quite a wide

impact range. It did depend on the cone angle however, and Cameron decided that a 90° cone gave results that represented the grease hardness accurately.

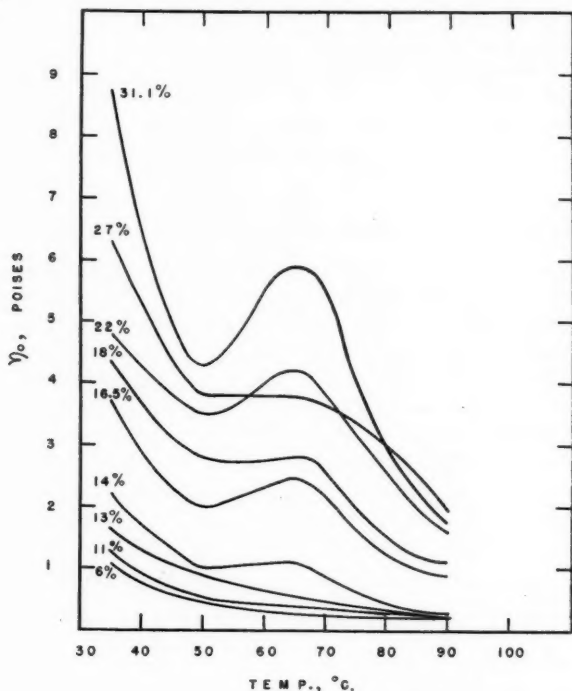


FIG. 6. Change in residual viscosity with temperature at different soap concentrations.

Since there should be a relation between hardness and the yield value of a grease as defined by Bingham (6), i.e., the shearing force per unit area necessary to overcome the internal elastic stress, the yield values of the present series of greases were determined by extrapolating the linear portions of the rate of shear vs. shearing stress curves, shown in Fig. 1, to zero rate of shear. The hardness values were determined as described by Cameron, using a 90° hollow cone with a 6 cm. slant height having a light graduated rod attached to the open end. The assembly weighed 78 gm. Extra weights were added to give penetrations of at least 10 mm. The assembly was allowed to fall from 5 to 25 cm.

Cameron's results were confirmed to the extent that the hardness of the grease was constant over the range of impact energies used up to a soap concentration of 25%. In Table II the hardness values are compared with the Bingham yield values and the coefficients of thixotropy.

TABLE II
COMPARISON OF HARDNESS AND THIXOTROPY OF GREASES

% Soap	H, dynes/cm. ²	θ , dynes/cm. ²	Yield value, dynes/cm. ²
13	25.1	5.6	5.0
14	26.6	6.1	5.5
16.5	49.4	9.7	10.0
18	71.8	22.0	20.2
22	113	44.2	41.7
26	149	74.2	76.0
31.1	406	145.2	133.5

The absolute values of the yield values agree well with coefficients of thixotropy, any difference probably being due to extrapolation error. This is to be expected since the Goodeve and Bingham equations are identical, with the exception of the difference in meaning attached to θ . Both may be expressed in dynes per square centimeter.

When the values in Table II are plotted (Fig. 7), the relation is approximately linear with $H = 20 + 2\theta$. This relation changes somewhat with cone

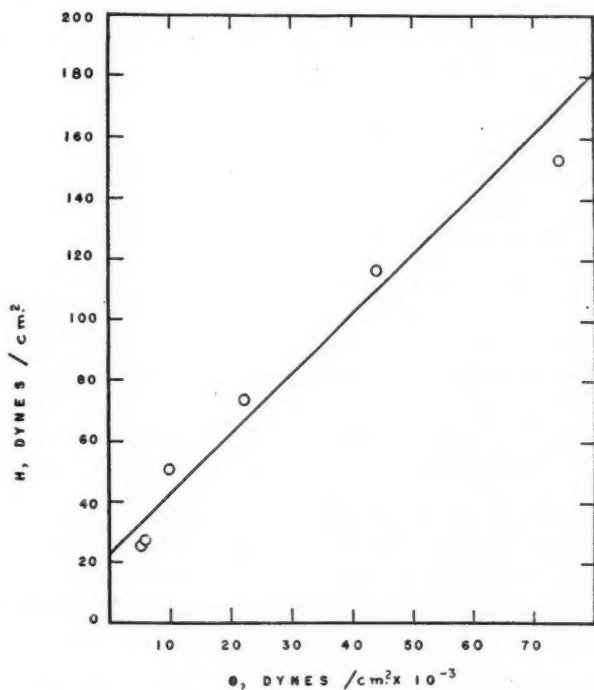


FIG. 7. Comparison of hardness and the coefficient of thixotropy.

angle and it might be possible to produce a cone that would give closer absolute values. It is also probable that this value depends on the particle shape of the solid phase and may have a different relation for other types of grease.

In grading lubricating greases the degree of thixotropy or yield value is of much more importance than the viscosity or mobility, since the latter property is controlled largely by the viscosity of the oil used in its manufacture. The usefulness of the finished product depends principally on the coefficient of thixotropy. The hardness test described by Cameron offers a rapid method of estimating this property and gives a result that is more clear-cut scientifically than the double cone penetrometer test commonly used today.

The results of the present investigation illustrate clearly that the short-coming of calcium base lubricating greases is due to an interaction between the soap and oil at a relatively low temperature, which seriously alters the flow characteristics, causing an increase in the apparent viscosity and almost complete loss of thixotropy. This change is not usually reversible and the value of the grease as a lubricant is greatly diminished. Previous investigations here and elsewhere (13, 22) would suggest that this is due to a phase change in the soap and does not depend on the oil used during the manufacture.

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THE USE OF P^{32} LABELED GLYCEROPHOSPHATE IN THE STUDY OF YOLK PHOSPHOLIPIDS¹

By J. W. T. SPINKS, C. C. LEE, AND J. B. O'NEIL

Abstract

Labeled sodium glycerophosphate has been prepared by phosphorylating glycerol with sodium dihydrogen phosphate containing P^{32} . After the labeled sodium glycerophosphate had been fed to a laying hen, radiophosphorus from glycerophosphate appeared in the shells, whites, and yolks of the eggs. The uptake in different eggs followed a pattern similar to that found for inorganic phosphate, the maximum occurring at the fourth or fifth egg. Comparative studies using P^{32} labeled sodium glycerophosphate and trisodium phosphate indicate that the rate of utilization of the phosphorus from glycerophosphate is less than the rate of utilization of the phosphorus from inorganic phosphate. The possibility of an enzymatic hydrolysis of glycerophosphate followed by the incorporation of the resulting phosphoric acid into the phospholipids and the phosphorylation of lower glycerides (partially hydrolyzed fat) by inorganic phosphate as a mechanism for phospholipid formation is discussed.

Introduction

The incorporation of phosphorus from inorganic phosphate into yolk phospholipids was first demonstrated by Hevesy and Hahn (14), and by Entenman *et al.* (9), through the injection of P^{32} labeled sodium phosphate into hens. It was concluded that the phospholipids are formed in the liver and carried by the blood plasma to the ovary, where their deposition in the yolks takes place (14). In 1942, Chargaff (6) administered P^{32} to hens by intramuscular injection of sodium phosphate, and then determined the specific activity of the phosphorus in the free and bound (with lipoproteins) lecithin and cephalin, the vitellin, and the acid soluble fraction from the yolks of eggs laid in the course of eight days following the injection. P^{32} containing ovovitellin prepared in this way has recently been applied by Francis and Wormald (12) to the immunological investigation of the vitellin-antivitellin precipitin reaction. Further applications of P^{32} in the study of hens' eggs has been reported by Lorenz *et al.* (16), who determined the per cent uptake of radiophosphorus in a large number of eggs from hens after injection of labeled sodium phosphate, and by O'Neil *et al.* (17), who determined the uptake of phosphorus in the shells, whites, and yolks of eggs from hens that received a diet in which the bone meal was replaced by calcium phosphate containing P^{32} .

In a review on phospholipid metabolism, Chaikoff (4) pointed out that consideration of the structural formulae suggests that glycerophosphate might act as intermediate in the formation of the phospholipids, lecithin and cephalin. The observation of Flock and Bollman (11) that the specific activity of glycerophosphate, isolated from cock livers after administration of P^{32} labeled sodium phosphate, was higher than that of the phospholipids and lower than that of

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inorganic phosphate from these livers is suggestive of such an intermediary role for glycerophosphate. Moreover, Zilversmit *et al.* (24) have, very recently, presented evidence to indicate that, after injection of inorganic phosphate into dogs, the specific activity of dog liver alkali-stable phosphorus, which was found to be rich in glycerophosphate, satisfied the criteria for a precursor of dog liver lecithin. No comprehensive study of the metabolic relationship between glycerophosphate and the phospholipids by the direct use of labeled glycerophosphate has, so far, been reported in the literature. Although Taurog and Chaikoff (4, 23) have isolated P^{32} labeled glycerophosphate from radioactive rat liver phosphatides and found that the activity from glycerophosphate did incorporate into the phospholipids of rat liver and kidney *in vitro* and *in vivo*, it was uncertain whether a breakdown of the glycerophosphate to inorganic phosphate occurred before the incorporation of the radiophosphorus into the phospholipids. The procedure for Taurog's method of preparation of labeled glycerophosphate was recently reported by Zilversmit *et al.* (24).

P^{32} labeled α -glycerophosphate has been prepared by Chargaff (5) according to the method of Fischer and Pfähler (10), which involved the treatment of isopropylidene glycerol with phosphoryl chloride containing P^{32} . Since P^{32} is generally obtained in the form of inorganic phosphate, a much simpler method is afforded by the well known commercial procedure for the preparation of glycerophosphate from glycerol and sodium dihydrogen phosphate (19). King and Pyman (15) have studied the constitution of this compound and concluded that it is the β isomer. Crystalline disodium glycerophosphate (crystallized from alcohol and water) has been prepared in this laboratory according to this procedure. It melted in the same range (85° to 95° C.) as the commercially obtained mixtures of α - and β -sodium glycerophosphate. It is therefore unlikely that the product is of the pure β form, although the β isomer may be the predominant one in the mixture of α - and β -sodium glycerophosphate we prepared. It was also found that a better yield was obtained when the product was isolated as barium glycerophosphate. The present report deals with experiments in which laying hens were given P^{32} labeled disodium glycerophosphate prepared by the phosphorylation of glycerol with radioactive sodium dihydrogen phosphate. Some of the hens were also given P^{32} labeled trisodium phosphate after the glycerophosphate activity had decayed to a negligible amount. A comparison of the utilization of phosphorus from glycerophosphate and inorganic phosphate in the formation of yolk phospholipids is thus possible.

Experimental

Synthesis of Glycerophosphate

Sodium dihydrogen phosphate (1.18 gm.) containing 76,900 registers per minute* of P^{32} activity was heated with 3 cc. of glycerol under reduced pressure

* One register per minute on the mechanical counter is equal to 128 radioactive disintegrations counted. The actual number of beta disintegrations is equal to the number of counts multiplied by a factor of 3.9 (counter efficiency was measured using a RaD-RaE standard from the National Bureau of Standards, Washington).

at 180° to 190° C. for two hours. The resulting mixture was refluxed for two hours with 20 cc. of 10% sodium hydroxide solution and then a barium chloride solution was added to precipitate all unreacted inorganic phosphate. Following the removal of the barium phosphate, it was found that some of the barium glycerophosphate could be precipitated out by bringing the solution to boiling. However, a more complete recovery of the glycerophosphate was effected by the method of Rae (20), which involved the precipitation of lead glycerophosphate, the decomposition of the lead salt with hydrogen sulphide, the removal of lead sulphide and excess hydrogen sulphide, the neutralization of glycerophosphoric acid with barium hydroxide, and then the precipitation of barium glycerophosphate with alcohol. The yield based on the P^{32} activity was 65.3%.

Analysis $C_3H_7O_6P\text{Ba}$ requires $P = 10.08\%$. Found $P = 9.85\%$.

For the experiments described below, weighed amounts of barium glycerophosphate were dissolved in water and the barium ions were removed by precipitation with equivalent amounts of sodium sulphate.

One of the main sources of phosphorus in the laying mash fed to our experimental birds is the bone meal, which contains about 140 mgm. of phosphorus in a single day's feed. To make it possible to compare the results of the present experiments with those of previous experiments with calcium phosphate (17), it was decided to replace the bone meal with sodium glycerophosphate and to administer to each hen an amount (active + inactive) equivalent to 140 mgm. of phosphorus.

Experiment A

A solution of sodium glycerophosphate, containing 140 mgm. of phosphorus with 3510 reg. per min. of P^{32} activity (0.79 microcurie) on the day of feeding, was mixed with the laying mash and fed to a Barred Plymouth Rock Pullet (Hen No. 1). Eighteen eggs laid subsequent to the feeding were separated into shells, whites, and yolks, then wet ashed with nitric and perchloric acid and analyzed for total activity according to the method of O'Neil *et al.* (17).

Experiment B

Two Barred Plymouth Rock Pullets (Hens No. 2 and 3) were isolated for this experiment. Each of these birds received 11 cc. of sodium glycerophosphate solution containing 140 mgm. of phosphorus with 7260 reg. per min. of P^{32} activity (1.63 microcurie) on the day of administration. This solution was injected subcutaneously into Hen No. 2 and was fed in a single feeding of laying mash to Hen No. 3. Each of the yolks of eggs No. 2, 3, 4, 5, 6 of both hens laid subsequent to the administration of the activity was dispersed in 100 cc. of 10% sodium chloride solution. A 10 cc. aliquot was wet ashed for determination of total activity. The remainder was extracted with three 80 cc. portions of ether, and the free (not bound to the lipoproteins) lecithin and cephalin fractions were isolated according to the method of Chargaff (6). The precipitated free lecithin and cephalin fractions were also wet ashed for determination of radioactivity. Appropriate aliquots of all ash solutions

were reserved for colorimetric determination of total phosphorus according to the method of Shelton and Harper (22).

Six 24 hr. samples of droppings from each hen were collected after the administration of the activity. These droppings were dried by hot air and then pulverized. To a representative 10 gm. aliquot, 200 cc. of distilled water was added and the mixture was allowed to sit overnight. The supernatant solution was then separated by centrifuge, and the residue washed thoroughly with distilled water. To the combined water extract and washings, barium chloride solution was added to precipitate the water soluble inorganic phosphates. Where necessary, ammonium hydroxide was added to render the solution slightly basic in order to get complete precipitation. This barium precipitate is called fraction A. It contains all water soluble inorganic phosphates. The filtrate from the barium precipitate is called fraction B. It contains all water soluble glycerophosphates. The residue from the original water extraction is called fraction C. It contains all water insoluble phosphorus compounds. After wet ashing, the radioactivity and total phosphorus of all fractions were determined.

Experiment C

Eighty days after the administration of labeled sodium glycerophosphate, it was found that the eggs of the hens used showed negligible activity. Hens No. 2 and 3 were then each given 10 cc. of trisodium phosphate solution containing 140 mgm. of phosphorus with 8280 *reg. per min.* of P^{32} activity (1.86 microcuries) on the day of administration. As in Experiment B, Hen No. 2 received the solution by subcutaneous injection and Hen No. 3 by feeding. The yolks of eggs subsequently laid were analyzed as in Expt. B.

Results

The results from Expt. A, expressed as % uptake of P^{32} , are shown by histogram in Fig. 1.

The activity per milligram of phosphorus for all the fractions from Expts. B and C can be calculated. This specific activity can be expressed as per cent of the initial specific activity, thus making possible the comparison of the results of experiments involving different amounts of radioactivity. The initial specific activity in these experiments is the activity administered to each hen divided by 140, the number of milligrams of phosphorus each hen received. The results are compared graphically in Figs. 2 and 3. It will be noted that our data may be made to agree with Chargaff's (6) if one assumes that in Chargaff's experiment each hen also received 140 mgm. of phosphorus in the feed.

Since the total activity of each yolk from Expts. B and C was determined, the total uptake of P^{32} can also be calculated. Results of these calculations are shown in Fig. 4.

Results of the determination of radioactivity in the droppings from Expt. B, expressed as per cent of P^{32} excreted, are tabulated in Table I.

TABLE I
EXCRETION OF P^{32} AFTER ADMINISTRATION OF SODIUM GLYCEROPHOSPHATE
SIX 24 HR. SAMPLES FROM EACH HEN

Sample No.	Fraction	Hen No. 2—Activity injected		Hen No. 3—Activity fed	
		% of P^{32} excreted	Total P, mgm.	% of P^{32} excreted	Total P, mgm.
1	A	16.40	191	16.68	201
	B	0.31	7.5	0.47	3.6
	C	8.75	243	9.56	198
2	A	3.74	198	4.53	231
	B	0.18	8.6	0.16	8.5
	C	1.19	209	2.12	193
3	A	0.95	193	1.78	116
	B	0.06	5.7	0.11	3.2
	C	0.87	171	1.27	182
4	A	0.82	204	1.00	160
	B	0.06	15.5	0.13	10.4
	C	0.94	292	0.53	279
5	A	1.06	143	0.45	210
	B	0.06	12.8	0.05	10.5
	C	0.63	362	0.38	274
6	A	0.84	166	0.09	48
	B	0.08	15.8	0.03	5.1
	C	0.28	234	0.04	227

Fraction A was water soluble and the activity was precipitated by barium ions.
Fraction B was water soluble but the activity was not precipitated by barium ions.
Fraction C was water insoluble.

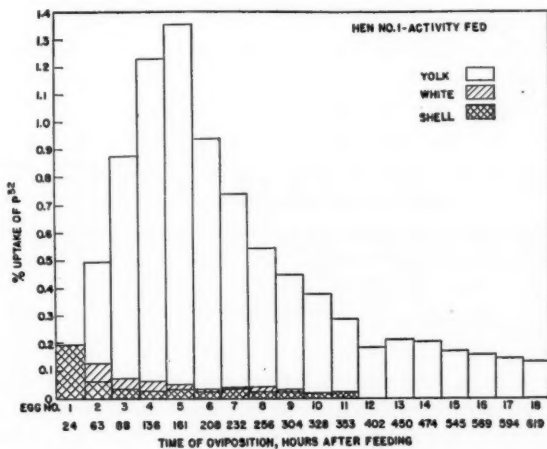


FIG. 1. Percentage uptake of P^{32} in successive eggs laid after feeding hen No. 1. with sodium glycerophosphate containing P^{32} .

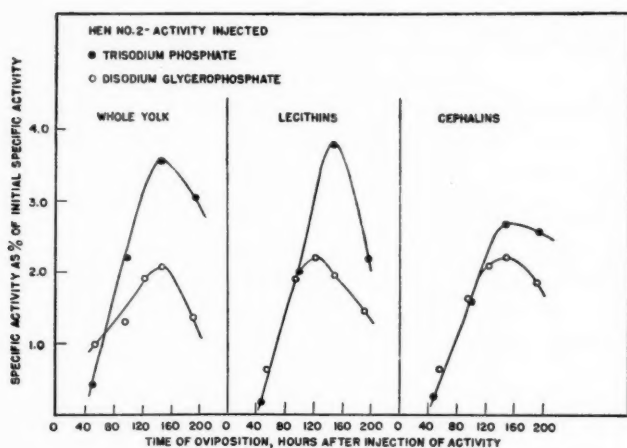


FIG. 2. Specific activities of whole yolk, lecithin, and cephalin from eggs of hen No. 2 injected (a) with sodium glycerophosphate and (b) eighty days later with sodium phosphate.

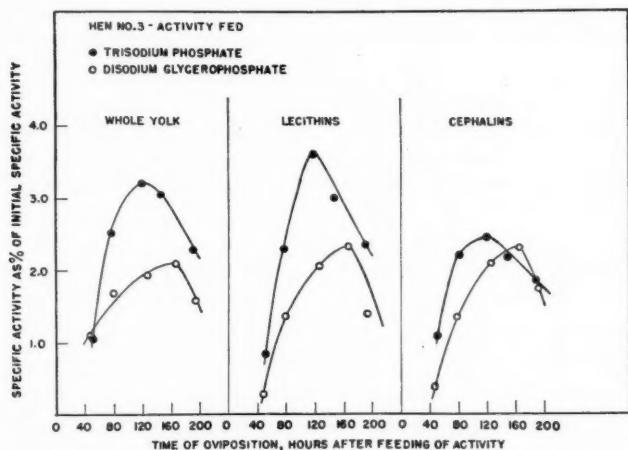


FIG. 3. Specific activities of whole yolk, lecithin, and cephalin from eggs of hen No. 3 fed (a) with sodium glycerophosphate and (b) eighty days later with sodium phosphate.

Discussion

It can be seen from Fig. 2 that the initial parts of the specific activity - time curves for the lecithin and cephalin fractions for trisodium phosphate and sodium glycerophosphate, administered by injection, coincided. In Fig. 3, where the data are from experiments in which the activities were administered by feeding, no such coincidence occurred. These variations may be due to individual differences of the experimental birds. With the limited number of

data, no significant conclusions can be drawn regarding the differences, if any, that arise from the different methods (injection and feeding) of administration of the labeled compound.

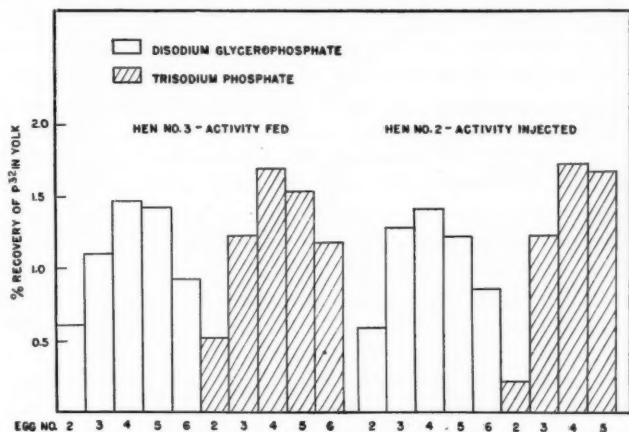


FIG. 4. Percentage recovery of P^{32} in yolks for hens No. 2 and 3 injected and fed, respectively, with sodium phosphate and sodium glycerophosphate.

A plot of specific activity against time is indicative of the rate of utilization of the phosphorus from the compound in question. Figs. 2 and 3 show that after administration of inorganic phosphate the maximum specific activities of the phosphorus in the whole yolk, lecithin, and cephalin fractions are higher than the corresponding maximum specific activities after administration of glycerophosphate. The total uptake of radiophosphorus from inorganic phosphate is also greater than the total uptake of radiophosphorus from glycerophosphate, as may be seen in Fig. 4. These results would seem to indicate that in the formation of yolk phospholipids, the rate of utilization of phosphorus from glycerophosphate is less than the rate of utilization of phosphorus from inorganic phosphate. The fact that after administration of glycerophosphate very little water soluble glycerophosphate was excreted as such (Table I) indicates that the bulk of the glycerophosphate was hydrolyzed before excretion. It is, of course, possible that the incorporation of the phosphorus from glycerophosphate into phospholipids results from the use of the phosphoric acid after the glycerophosphate is enzymatically hydrolyzed. However, these experiments provide no positive evidence on this point. Experiments with glycerophosphate labeled with both C^{14} and P^{32} would afford more conclusive proof as to whether this compound is hydrolyzed before utilization in the biosynthesis of phospholipids.*

* In view of the possible difference in rates of utilization of the α and β isomers, experiments with the pure isomers (including optically active ones) would also be of interest.

It is of some interest to note that experiments by other workers using P^{32} labeled phosphoryl ethanolamine (7) and phosphoryl choline (21) indicated that these esters are not used directly in phospholipid formation. Recently, Frazer and Sammons (13) obtained evidence indicating that fats are absorbed as lower glycerides (mono and di) and tentatively suggested that these lower glycerides form the basis for phospholipid synthesis. It could perhaps be further suggested that phosphorylation of the lower glycerides can be effected only by inorganic phosphate. In that case, the phosphorus from esters such as glycerophosphate, phosphoryl choline, and phosphoryl ethanolamine would be used by the animal for phospholipid synthesis only after hydrolysis to phosphoric acid. Reaction between a phosphorylated diglyceride and the nitrogen containing base would then give rise to the phospholipid molecule. Such a mechanism would account for the observation that ethanolamine and its products of partial methylation (2) as well as choline (1, 8, 18) stimulated the utilization of radiophosphorus from injected inorganic phosphate for phospholipid formation while, on the other hand, the utilization of radiophosphorus from labeled phosphoryl ethanolamine (7) and phosphoryl choline (21) was not as great as that from inorganic phosphate. Furthermore, it has been observed that the rate of formation of rat liver phospholipids was increased by a fat-rich diet (3). The stimulating effect of choline on lipid phosphorylation was also found to be enhanced by simultaneous ingestion of a large amount of fat (1). Such observations can be attributed to a greater availability of lower glycerides from a fat-rich diet if these lower glycerides were involved in phospholipid formation as required by the mechanism suggested above. The findings of Flock and Bollman (11) and Zilversmit *et al.* (24) can also be reconciled to the suggested mechanism of phospholipid formation if the glycerophosphate fractions obtained by these workers were derived from phosphorylated lower glycerides.

Conclusion

Studies using P^{32} labeled sodium glycerophosphate and sodium phosphate indicate that the rate of utilization of the phosphorus from glycerophosphate is less than the rate of utilization of the phosphorus from inorganic phosphate. To account for this and other observations reported in the literature, it is suggested that organic phosphate esters are not used directly in phospholipid formation. They may, however, be first hydrolyzed enzymatically and the resulting phosphoric acid may then be incorporated into the phospholipid molecule. The phosphorylation of lower glycerides (partially hydrolyzed fats) solely by inorganic phosphate is tentatively suggested as an essential step in the biosynthesis of the phospholipids, lecithin and cephalin.

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SOLUBILITY OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN ORGANIC SOLVENTS AT 20° C.¹

BY L. YAFFE²

Abstract

A study has been made of the solubility of uranyl nitrate hexahydrate and thorium nitrate tetrahydrate in various organic solvents at 20° C. In all the solvents investigated no case was found where the thorium nitrate tetrahydrate was more soluble than the uranyl nitrate hexahydrate. Hydrocarbons do not dissolve either compound to an appreciable extent. Addition of a ketone, aldehyde, or alcohol group enhances the solubility considerably. The simpler the compound the greater the solubility of the nitrate in any given family.

Introduction

In order to establish a general relationship between the solubilities of the nitrates of some of the heavier elements in organic solvents and the molecular constitution of the solvent, and to supplement the very meager quantitative data existing, a comprehensive survey of the solubilities of uranyl nitrate hexahydrate, $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and thorium nitrate tetrahydrate, $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, was made in organic solvents that were available. At the time this work was undertaken no such data were available.

Wells (10) measured the solubility of thorium nitrate in ether to determine whether Hillebrand's (3) method of dissolving uranyl nitrate in ether in order to remove last traces of rare earths was applicable in this case. Misciattelli (6) measured the solubilities of uranyl nitrate and thorium nitrate in ether. In order to get data about the quaternary system uranyl nitrate - thorium nitrate - ether - water Misciattelli (7, 8) also studied the two ternary systems, uranyl nitrate - ether - water and thorium nitrate - ether - water. Imre (4, 5) extracted thorium nitrate from aqueous solution using ether. Mlle O. Guempel (2) also studied the system uranyl nitrate - ether - water. Gmelin (1) lists qualitative data regarding a few solvents in which uranyl nitrate is soluble. Templeton and Hall (9) have recently published a comprehensive survey of the solubility of thorium nitrate tetrahydrate at 25° C.

Experimental

(a) Materials

Thorium nitrate tetrahydrate Bakers C.P. analyzed was used in all cases. Uranyl nitrate hexahydrate was obtained from the Eldorado Mining and Refining Co. The maximum impurities consisted of rare earths (<0.1%). The organic solvents used were "practical" grade or better.

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(b) *Method*

Twenty-five cubic centimeters of the solvent was used in each case. To this volume in a 50 cc. centrifuge tube was added a few grams of thorium nitrate tetrahydrate or uranyl nitrate hexahydrate. The tube was then sealed and agitated in "end-over-end" fashion in a water bath thermostatically controlled at $20^{\circ}\text{C.} \pm 0.5^{\circ}$. More solid was added if necessary until a saturated solution was obtained. The minimum time for saturation to occur was taken to be 24 hr. Some samples were agitated for four days before saturation was complete. Some samples showed a discoloration that was probably due to solvent decomposition. In order to ensure that true equilibrium existed, several solvents that did not decompose were tested at higher temperatures and allowed to cool down to 20°C. Analysis of the solution showed good agreement between results obtained by agitation at 20°C. and by approaching the equilibrium from a higher temperature. Before analysis, the solution was centrifuged to remove any of the solid that might have been present in the form of a suspension.

To analyze for thorium, an aliquot of the solvent layer was pipetted off, the solvent evaporated off, and the residue ignited to thorium oxide.

The uranyl nitrate was back-extracted into water and the concentration determined colorimetrically. The solution was diluted sufficiently so that the concentration was in the range where Beer's law held. Results of these analyses were checked using U^{233} as tracer. The samples were counted using an ionization chamber and linear amplifier (11) and were found to agree with the colorimetric analyses to within $\pm 5\%$ and all analyses are quoted to within that figure.

Results

The solubilities of uranyl nitrate hexahydrate and thorium nitrate tetrahydrate in various ethers is given in Table I. The results are shown graphically in Fig. 1 where the change in solubility with increasing number of carbon atoms is plotted for straight chain aliphatic ethers.

In Table II and Fig. 2 are shown the results for alcohols, with a graphical representation of the solubility in straight chain aliphatic alcohols.

In Table III are shown the solubilities in the various esters. In Fig. 3 is shown the variation of the solubility of uranyl nitrate hexahydrate and thorium nitrate in various acetates with increasing number of carbon atoms. The results for homologous series of amyl esters are also plotted.

Tables IV, V, VI, VII list the results of the solubilities in various ketones, aldehydes, hydrocarbons, and substituted hydrocarbons respectively.

Some generalizations may be drawn from the data presented.

1. In all the solvents examined, where solubility could be measured, no case has been found where the solubility of thorium nitrate tetrahydrate in a selected solvent is greater than that of uranyl nitrate hexahydrate.
2. Hydrocarbons do not dissolve either compound to any appreciable extent.

TABLE I

SOLUBILITY OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS ETHERS AT 20° C. IN ORDER OF DECREASING SOLUBILITY

Solvent	UO ₂ (NO ₃) ₂ · 6H ₂ O, gm. per 100 cc. of solvent	Th(NO ₃) ₄ · 4H ₂ O, gm. per 100 cc. of solvent
Diethylcellosolve	102	6.8
Dimethyldioxane	92	30.0
Diethyl ether	74	17.0
Ethylhexylcellosolve	68	6.9
Dibutylcellosolve	49	6.4
Dibutylcarbitol	44	8.8
Pelargonic ether	17	—
<i>n</i> -Butyl ether	16	—
Xanthyl ether	14	—
Amyl ether	11	.02
Hexyl ether	9	0.08
Isopropyl ether	9	—
Dichloroisopropyl ether	5	0.4
Isoamyl ether	<0.1	<0.1
Amylphenyl ether	"	"
<i>p</i> -tert-Amylphenyl methyl ether	"	"
β -Bromoethyl ethyl ether	"	"
γ -Bromopropyl phenyl ether	"	"
Methyl phenyl ether	"	"
<i>o</i> -Chloroisopropyl ether	"	"
2-Chloro-4-tert-amyl phenyl ether	"	"
<i>m</i> -Cresyl methyl ether	"	"
<i>o</i> -Cresyl methyl ether	"	"
<i>p</i> -Cresyl methyl ether	"	"
$\beta\beta'$ -Dichloroisopropyl ether	"	"
$\beta\beta'$ -Dichloroethyl ether	"	"
$\gamma\gamma$ -Dichloroisopropyl ether	"	"
1-8 Epoxy <i>p</i> -menthane	"	"
Eugenol methyl ether	"	"
Resorcinol dimethyl ether	"	"

TABLE II

SOLUBILITIES OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS ALCOHOLS AT 20° C. IN ORDER OF DECREASING SOLUBILITY

Solvent	UO ₂ (NO ₃) ₂ · 6H ₂ O, gm. per 100 cc. of solvent	Th(NO ₃) ₄ · 4H ₂ O, gm. per 100 cc. of solvent
Dibutoxytetraethylene glycol	90	75.9
Isobutylcarbinol	57	30.0
Diethylcarbinol	57	9.1
<i>n</i> -Amyl alcohol	55	7.3
2-Ethyl butyl alcohol	49	9.0
Methyl amyl alcohol	43	—
<i>n</i> -Heptyl alcohol	43	—
Capryl alcohol	37	8.1
Geraniol	36	—
β, γ -Dibromopropyl alcohol	32	4.8
Undecanol	27	5.5
Tetradecanol	13	0.37

3. Addition of a ketone, aldehyde, or alcohol group enhances the solubility considerably.
4. For a given family the solubility decreases with increasing complexity of the molecule.

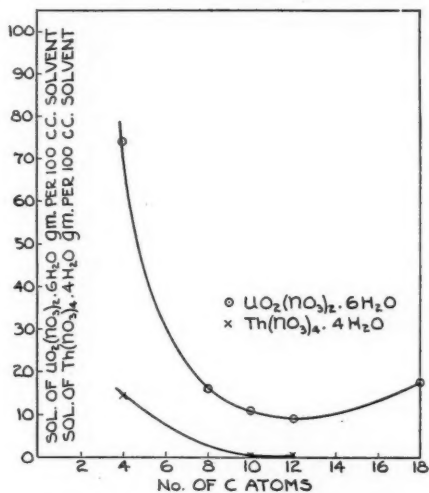


FIG. 1. Relation between solubility of uranyl nitrate hexahydrate and thorium nitrate tetrahydrate and number of C atoms in straight chain aliphatic ethers.

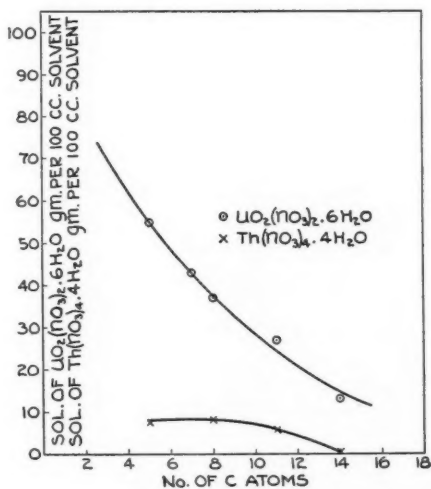


FIG. 2. Relation between solubility of uranyl nitrate hexahydrate and thorium nitrate tetrahydrate and number of C atoms in straight chain aliphatic alcohols.

TABLE III

SOLUBILITIES OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS ESTERS AT 20° C. IN ORDER OF DECREASING SOLUBILITY

Solvent	$\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, gm. per 100 cc. of solvent	$\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, gm. per 100 cc. of solvent
β -Ethoxyl ethyl acetate	125	—
Ethyl acetylglycollate	110	30.0
Ethyl acetate	82	—
Butylcellosolve acetate	77	—
Butyl acetate	68	6.5
Isopropyl acetate	64	20.0
sec-Butyl acetate	61	9.0
Amyl formate	56	30.0
<i>n</i> -Butyl propionate	55	27.5
Isoamyl acetate	55	18.0
Isobutyl acetate	50	16.0
Ethyl sebacate	48	—
Amyl acetate	46	9.2
Butyl adipate	40	—
<i>n</i> -Butyl sebacate	37	2.1
<i>n</i> -Amyl propionate	37	—
β -Phenyl ethyl acetate	35	—
Methyl phenyl acetate	33	—
Isobutyl propionate	31	—
Vinyl acetate	31	2.5
Isoamyl propionate	27	—
Amyl succinate	25	—
Amyl butyrate	25	4.3
Isoamyl formate	24	20.0
Amyl valerianate	21	4.2
Geraniol acetate	16	—
<i>n</i> -Butyl oxalate	9	0.03
Isoamyl <i>n</i> -caproate	7	2.4
Isoamyl oxalate	< 0.1	< 0.1
Benzyl <i>n</i> -butyrate	"	"
Butylcellosolve oleate	"	"
<i>n</i> -Butyl salicylate	"	"
Ethyl laureate	"	"
Ethyl myristate	"	"
Linalyl acetate	"	"
Phenyl ethyl benzoate	"	"
Isopropyl benzoate	"	"
<i>n</i> -Butyl nitrate	"	"
Diphenyl mono(<i>p</i> -tert-butylphenyl) phosphate	"	"
Di(<i>p</i> -tert-butylphenyl) monophenyl phosphate	"	"
Di(<i>o</i> -chlorophenyl) monophenyl phosphate	"	"

TABLE IV

SOLUBILITIES OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS KETONES AT 20° C. IN ORDER OF DECREASING SOLUBILITY

Solvent	$\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, gm. per 100 cc. solvent	$\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, gm. per 100 cc. solvent
Cyclohexanone	105	—
Methyl ethyl ketone + 15% xylene	100	75.0
Diethyl ketone	76	—
Methyl isobutyl ketone	75	26.0
Methyl <i>n</i> -amyl ketone	68	17.0
Diisopropyl ketone	41	6.6

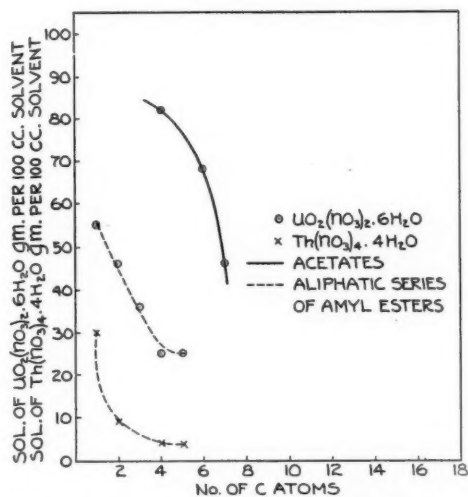


FIG. 3. Relation between solubility of uranyl nitrate hexahydrate and thorium nitrate tetrahydrate and number of C atoms in various aliphatic esters.

TABLE V

SOLUBILITIES OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS ALDEHYDES AT 20° C.

Solvent	$\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, gm. per 100 cc. solvent	$\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, gm. per 100 cc. solvent
Acetaldehyde	42	—
Citronellal	34	—
Anisaldehyde	27	—
<i>n</i> -Butyraldehyde	22	0.49

TABLE VI

SOLUBILITIES OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS HYDROCARBONS AT 20° C.

Solvent	UO ₂ (NO ₃) ₂ · 6H ₂ O, gm. per 100 cc. solvent	Th(NO ₃) ₄ · 4H ₂ O, gm. per 100 cc. solvent
<i>x</i> -Triisopropyltoluene	<0.1	<0.1
1-Pentene	"	"
Benzene	"	"
<i>p</i> -Isopropyltoluene	"	"
Linalool	"	"
Isopentane	"	"
Petroleum ether	"	"
Toluene	"	"
Turpentine	"	"
Xylene	"	"

TABLE VII

SOLUBILITIES OF URANYL NITRATE HEXAHYDRATE AND THORIUM NITRATE TETRAHYDRATE IN VARIOUS SUBSTITUTED HYDROCARBONS AT 20° C.

Solvent	UO ₂ (NO ₃) ₂ · 6H ₂ O, gm. per 100 cc. solvent	Th(NO ₃) ₄ · 4H ₂ O, gm. per 100 cc. solvent
Nitromethane	64	0.04
Triglycol dichloride	57	8.2
1-Nitropropane	8	—
2-Nitropropane	4	—
Nitrobenzene	1.5	—
Tetrabutylurea	6.0	6.0
Isoamyl bromide	< 0.1	<0.1
<i>m</i> -Chloroethylbenzene	"	"
<i>x</i> -Dichloro- <i>x</i> -diethylbenzene	"	"
Benzyl chloride	"	"
Carbon tetrachloride	"	"
1-Chloro-1-nitropropane	"	"
2-Chloro-2-nitropropane	"	"
1-Chloro-1-nitroethane	"	"
Chloroform	"	"
Dichlorobenzene	"	"
1,1-Dichloro-1-nitropropane	"	"
Ethylene bromide	"	"
Ethylene iodide	"	"
<i>s</i> -Tetrabromomethane	"	"
<i>s</i> -Tetrachlorethane	"	"
Trichlorethylene	"	"
Diethylamine	"	"
Diethyleneamine	"	"
Hydroxyethylethylenediamine	"	"
Triamylamine	"	"
Triethanolamine	"	"
Piperidine	"	"
Quinoline	"	"
β -Hydroxy- <i>o</i> -toluidine	"	"
2-Bromopyridine	"	"
Diamylphenol	"	"
2-Chloro-4,6-di- <i>tert</i> -amylphenol	"	"

Acknowledgments

The author wishes to acknowledge the assistance of J. Hebert and Miss C. E. Mackintosh in carrying out some of the analyses quoted above.

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THE COLORIMETRIC DETERMINATION OF SOME SYNTHETIC OESTROGENS¹

BY R. A. DUNFORD

Abstract

The synthetic oestrogens, stilboestrol, hexoestrol, and dienoestrol give color reactions with nitric and nitrous acids, bromine, and certain phenol reagents, some of which can be adapted to their colorimetric determination. The use of Folin and Ciocalteu's phenol reagent is described for determination of the hormones in pharmaceutical products, using a Lumetron photoelectric colorimeter. The interference of other oestrogens and of phenolic preservatives and bactericides is considered and reference is made to a means of identifying the hormones by color reactions with antimony pentachloride in ethylene dichloride, and with an acetic-phosphoric acid reagent.

Introduction

The synthetic sex hormones, stilboestrol (I), hexoestrol (II), and dienoestrol (III) are all hydroxy derivatives of stilbene (IV) and vary only in the number of olefin linkages in the side chains. Hexoestrol is saturated, stilboestrol has one unsaturated linkage, and dienoestrol has a diene configuration (Fig. 1).

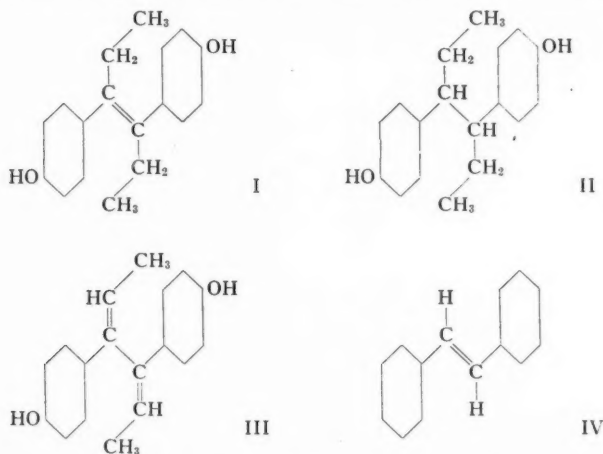


FIG. 1. Structural formulae of the hormones stilboestrol (I), hexoestrol (II) and dienoestrol (III), and of the parent compound stilbene (IV).

In the determination of these compounds in pharmaceutical products in which the dosage is of the order of from 0.1 to 5.0 mgm. a sensitive colorimetric procedure is required, and a number of methods of estimation have been suggested based either upon oxidation or bromination at the double bonds with

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formation of colored products or upon reaction of the phenolic groups with various phenol reagents.

Malpress's method (5) is based upon measurement of the orange color produced when a solution of the hormone in glacial acetic acid is treated with a small quantity of concentrated sulphuric and nitric acids and subsequently rendered alkaline with ammonia. This method has the disadvantage of low sensitivity—being unable to detect less than 0.5 mgm. of hormone, and is not specific, as many other organic compounds produce colors under these conditions.

Cocking (2) developed a color reaction for stilboestrol and dienoestrol based on bromination of the hormone in acetic acid solution and removal of excess bromine with phenol. This gives a green color on subsequent heating but it is suitable only as a qualitative test, for the shade as well as depth varies with concentration.

With a large excess of bromine a purple color is produced upon subsequent dilution with water; this color is apparently due to a colloidal compound, and it may be stabilized by gelatin. This reaction may be used as a basis for estimation of the pure hormones, but mere traces of fatty matter or stearates from oil solutions or tablets will inhibit the reaction.

Gottlieb's method (3, 4) depends upon the conversion of the oestrogen to the corresponding *o*-nitrosophenol by the action of nitrous acid, and the color measurement of the quinoid form in alkaline solution.

Experimental

The work in our laboratory upon the establishment of a standard method of estimation has been based upon the investigation of reaction of the hormones with the three commonly used phenol reagents, Gibbs's dibromoquinone-chloroimide reagent, Folin and Denis's reagent, and Folin and Ciocalteu's reagent.

It was found that Gibbs's reagent gave no color reaction with any of the three hormones; this bears out the theory that it does not react with *p*-substituted phenols. Folin and Denis's reagent is the molybdate-tungstate reagent specified for the assay of stilboestrol in the B.P. and U.S.P., while Folin and Ciocalteu's reagent is a modification of the above, containing lithium sulphate.

Both reagents are reduced by phenolic compounds, giving the molybdenum blue complex in alkaline solution. This complex has a rather flat spectral absorption curve; but measurements may best be carried out at 640 m μ . Absorption curves plotted for the blue color, using 0.2 mgm. and 0.5 mgm. of stilboestrol, showed that the wave length giving maximum sensitivity was 640 m μ .

The colors produced by the hormones with both Folin and Denis's, and Folin and Ciocalteu's reagent show conformance to Beers' law (Fig. 2) but a

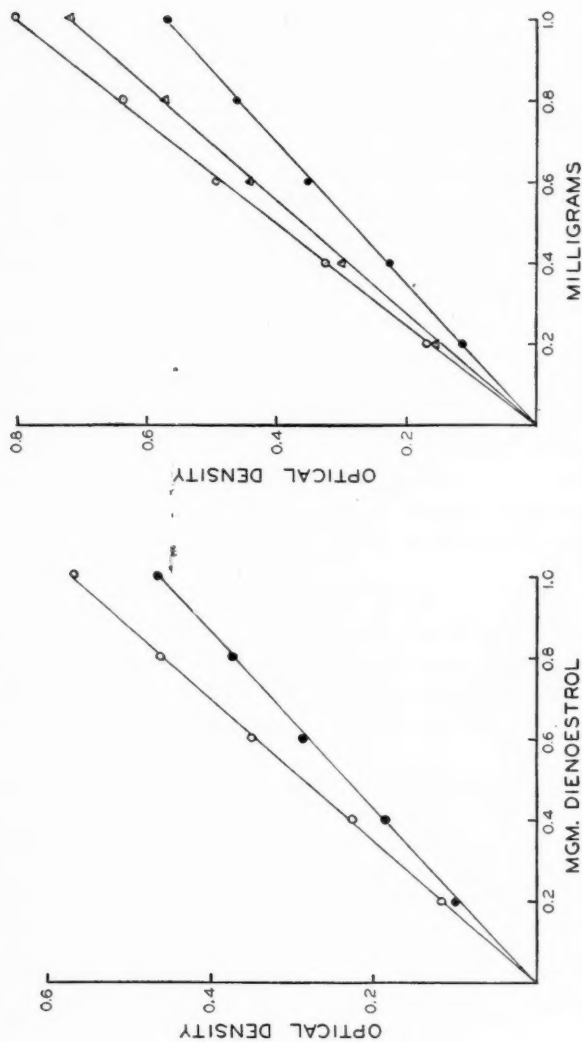


FIG. 2. Calibration curves for dienoestrol using Folin and Denis's reagent (solid circles) and Folin and Ciocalteu's reagent (clear circles). Abscissae represent mgm. sample per 5 ml. of solution taken.

FIG. 3. Calibration curves for hexoestrol (clear circles), stilboestrol (triangles), and dienoestrol (solid circles), using Folin and Ciocalteu's reagent. Abscissae represent mgm. sample per 5 ml. of solution taken.

steeper curve was given by Folin and Ciocalteu's reagent, indicating a somewhat greater sensitivity. Both reagents show a tendency to discolor upon storage, becoming dark green and unfit for use. Folin and Ciocalteu's reagent can, however, be readily regenerated by raising to boiling point, adding just sufficient bromine water dropwise to restore the full yellow color, boiling off any excess of bromine, and making up to its original volume on cooling. In view of this, Folin and Ciocalteu's was the reagent adopted for use, and standard curves were prepared for routine estimation of the three hormones on a Lumetron No. 402 EF photoelectric colorimeter, using filter M640.

Solutions of the hormones in 50% methanol (reagent grade) were prepared with concentrations ranging from 0.04 to 0.2 mgm. per ml., and 5 ml. portions of these solutions were pipetted into 100 ml. volumetric flasks. Five milliliters of the reagent was next added, and after standing for five minutes the solution was made alkaline by addition of 25 ml. of 10% sodium carbonate, well shaken, made up to 100 ml. with water, and allowed to stand for one hour for the color to develop. The solutions were centrifuged to remove the precipitate formed, and the clear liquid transferred to 13 mm. absorption cells. The percentage transmittance of each solution was measured, after setting the instrument for 100% transmittance on a blank prepared by treating 5 ml. of 50% methanol with the reagents in the same manner (Fig. 3).

The use of Folin and Ciocalteu's reagent has been applied to the determination of the hormones in tablets, ampoules, and ointment bases as follows.

Determination in Tablets

The uncoated tablets are crushed and a weighed quantity equivalent to about 5 mgm. of the oestrogen is extracted by maceration with ether.

Coated tablets should preferably have the color coat removed by agitating in a Gooch crucible with water until the dye coat is removed, and may then be extracted similarly to the uncoated tablets.

The ether extract is evaporated and the residue dissolved in 50 ml. of methanol and made up to 100 ml. with water. A 10 ml. portion is taken, the Folin and Ciocalteu's reagent added, made alkaline with 25 ml. of 10% sodium carbonate, and diluted to 100 ml. After standing one hour the solution is centrifuged, and its percentage transmittance measured in the colorimeter and compared to the standard curve.

Determination in Ampoules

Oil solutions of the hormones may be assayed by dilution of a measured volume with ether and extraction four times with *N*/1 sodium hydroxide. The combined alkaline extracts are acidified with dilute sulphuric acid and extracted five times with ether. The ether is then evaporated, the residue dissolved in alcohol, and the estimation carried out as for the tablets.

Determination in Ointment Bases

The oestrogens are sometimes compounded in ointment bases, or gynecological creams, and they may be estimated as for ampoules after first breaking down the emulsion by boiling under reflux with dilute hydrochloric acid and chloroform.

Five grams of the ointment or cream is dispersed in 25 ml. of water and 5 ml. of 5 *N* hydrochloric acid and 5 ml. of chloroform added. The mixture is refluxed for 20 min. or until a clear separation is obtained.

The chloroform layer is separated, and the aqueous layer washed with two further 10 ml. portions of chloroform. The bulk chloroform extracts are washed with two 5 ml. portions of water to remove traces of acid, and evaporated on a water bath. The residue is dissolved in ether and extracted with *N*/1 sodium hydroxide as for ampoules.

Results of some typical analyses of the above products are shown in Table I.

TABLE I

TYPICAL ANALYSES OF PHARMACEUTICAL PREPARATIONS OF SYNTHETIC OESTROGENS USING FOLIN AND CIOCALTEU'S PHENOL REAGENT

Preparation	Declared strength	Amount found, mgm.	Percentage found of declared amount
Dienoestrol tablets	0.3 mgm. per tablet	0.30	100
" "	0.3 " " "	0.29	96.5
" "	0.3 " " "	0.295	98
" "	1.0 " " "	0.96	96
" "	1.0 " " "	0.98	98
Stilboestrol "	1.0 " " "	0.97	97
" "	1.0 " " "	0.98	98
" "	1.0 " " "	0.96	96
" "	5.0 " " "	5.00	100
" "	5.0 " " "	4.90	98
Stilboestrol amps.	1.0 mgm. per cc.	0.98	98
" "	1.0 " " "	1.03	103
" "	5.0 " " "	5.10	102
Dienoestrol ointment	2.0 mgm. per fl. oz.	1.90	95
" "	5.0 " " "	4.85	97

Interference of Other Compounds

The reaction is not, of course, specific for the synthetic oestrogens, and is given by other phenolic compounds. Many of the preservatives for creams contain phenolic groups and a number have been investigated to determine their interference in the reaction.

It was found that while phenol and chlorocresol gave intense colors at low concentrations, the methyl and propyl *p*-hydroxy benzoates and phenylmercuric nitrate gave no color reaction in the concentrations in which they would be likely to be used as preservatives (Table II).

TABLE II

REACTION OF CERTAIN FUNGICIDES AND BACTERICIDES WITH FOLIN AND CIOCALTEU'S REAGENT

Compound	Concentration per 10 ml. sample, mgm.	Reaction	Per cent transmittance of solution
Chlorocresol	0.1	Positive	19
Phenol	0.1	Positive	15
Methyl- <i>p</i> -hydroxy benzoate	0.1	Negative	98
Propyl- <i>p</i> -hydroxy benzoate	0.1	Negative	98.5
Phenylmercuric nitrate	0.1	Negative	100.0
Stilboestrol (inserted for comparison)	0.02		66.0

Esters of the hormones, such as stilboestrol dipropionate, will not give a reaction unless they are first hydrolyzed, as they possess no free hydroxyl group. Similarly the natural oestrogen, oestradiol, gives a reaction, but its benzoic ester does not. The other natural sex hormones, progesterone and testosterone, are ketonic and give no color reaction.

Identification of the Hormones

The color reaction with Folin and Ciocalteu's reagent is given with hexoestrol, dienoestrol, and stilboestrol, and cannot be used to differentiate between them. Identification may be carried out by determination of the melting points of the separated hormones, which are:— stilboestrol, 170° C.; hexoestrol, 187° C.; dienoestrol, 233° C.

Two color reactions have also been published which can be used as a means of identification. Warren, Goulden, and Robinson (6) have found that solutions of stilboestrol and dienoestrol in ethylene dichloride will react with a 50% *V/V* solution of antimony pentachloride in the same solvent, to produce a red coloration. Hexoestrol gives no reaction other than a transient yellow tint. It should be noted that many other stilbene derivatives also give colors with this reagent.

A means of differentiating dienoestrol from the other oestrogens has been given by Boscott (1). The hormone, when dissolved in a few drops of glacial acetic acid and mixed with 1.8 ml. of 85% phosphoric acid, gives a sequence of colors from yellow to pink. On subsequent heating for one hour, adding 3 ml. of glacial acetic acid, and reheating, a purple coloration with a deep blue fluorescence is produced. Hexoestrol and dienoestrol do not give this reaction, but isodienoestrol gives a similar color.

Acknowledgment

The author is indebted to the directors of The British Drug Houses (Canada) Ltd. for permission to publish these results.

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THE ALKALOIDS OF PAPAVERACEOUS PLANTS

XLII. *DENDROMECON RIGIDA* BENTH.¹

BY RICHARD H. F. MANSKE

Abstract

Aside from small uncrystallized residues the only alkaloids found in *Dendromecon rigida* Benth. are protopine and allocryptopine.

Since there appeared to be no record of the chemical examination of *Dendromecon rigida* Benth. it seemed important to determine the nature of the contained alkaloids. The plant is a shrub native to the southwestern coast of North America and is the only species generally recognized in the genus *Dendromecon* Benth.

While the quantity available was inadequate for a thorough study it can be stated that only two alkaloids, namely protopine and allocryptopine, are present in more than traces. A very small phenolic fraction yielded traces of a crystalline oxalate of doubtful purity.

Experimental

There was available one kilo of dried leaves and young shoots, which was ground and extracted with methanol in a Soxhlet apparatus. The solvent was removed from the extract and the residue digested with very dilute hydrochloric acid. After cooling, the pale yellow aqueous solution was decanted from the resinous material through a layer of charcoal. The clear filtrate was extracted with chloroform until the extract was colorless. Examination of the chloroform yielded only a trace of protopine.

Protopine

The aqueous solution which had been extracted with chloroform was basified with ammonia and again extracted with chloroform. The residue from the chloroform extract was dissolved in dilute hydrochloric acid, and the solution filtered with the aid of charcoal, and treated with an excess of sodium hydroxide. The precipitated base was filtered off, washed, and dried. (When the alkaline filtrate was saturated with carbon dioxide and exhausted with ether it yielded a trace of protopine and a small amount of an amorphous base which afforded a quantity of a crystalline oxalate insufficient for further examination.) The dried base was dissolved in hot methanol, filtered, and evaporated to a small volume. Seeding with a crystal of protopine yielded an immediate crop of protopine weighing 0.6 gm. After one recryst-

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Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada, and from the Research Laboratories, Dominion Rubber Co. Ltd., Guelph, Ontario. Issued as N.R.C. No. 1973.

tallization from chloroform-methanol it melted sharply at 210° C.* either alone or in admixture with an authentic specimen of protopine.

Allocryptopine

The methanolic filtrate from the protopine was concentrated to a thin syrup and seeded with a crystal of allocryptopine. The base, which crystallized immediately (0.2 gm.), was recrystallized from hot methanol; it then melted at 160° C. either alone or in admixture with authentic allocryptopine.

The final mother liquor from the above alkaloids on further examination yielded only small amounts of the above two alkaloids.

* *Melting points are corrected.*

DINITRATION AND DEALKYLATION OF *p*-CYMENE¹BY O. C. W. ALLENBY² AND R. V. V. NICHOLLS

Abstract

An investigation of the dinitration of *p*-cymene (4-isopropyltoluene) has been carried out, with particular regard to determining the effects of varying the temperature, the nitric acid, sulphuric acid, and water contents of the system, the rate of addition of *p*-cymene, and the stirring time upon the yields of 2,6-dinitrocymene and 2,4-dinitrotoluene. Optimum nitrating conditions have been determined and the relative constancy of the dinitrocymene : dinitrotoluene ratio over a wide range of conditions has been established. Yields of the order of 55% dinitrocymene and 25% dinitrotoluene have been obtained. Various theories are offered with a view to explaining some of the more important observations.

Introduction

The action of nitrating mixtures on *p*-cymene (1-methyl-4-isopropylbenzene) has been fairly extensively investigated by a number of workers. Mono-, di-, and tri-nitrated products can be obtained by varying the reaction conditions. Attempts at mononitration have involved the addition of nitric acid to cymene (4, 10, 11, 19), addition of cymene to mixed nitric and sulphuric acids (31, 32), and addition of "mixed acid" to a mixture of cymene, sulphuric acid, and acetic acid (15, 21, 16, 28). The last method has been perfected by Doumani and Kobe (8) and results in consistent yields of 86 to 90% 2-nitrocymene and 8 to 10% 4-nitrotoluene (by loss of the isopropyl group). Wide variations in nitric acid, sulphuric acid, and acetic acid content, time of addition, and presence of emulsifying agents do not alter these values appreciably.

Dinitration of cymene has been achieved by the addition of cymene to nitric acid (3, 12, 31), and by the addition of cymene to mixed acid (1, 3, 15, 17, 18, 29, 31, 32). To Alfthan (1) goes the credit for the conclusive identification of the products, which were shown to be principally 2,6-dinitrocymene and 2,4-dinitrotoluene. He also devised a method for separating them by recrystallization from ethanol. From none of this work is it possible to draw accurate conclusions concerning the yields of the two principal products or the effect of reaction variables on these yields.

Trinitration of *p*-cymene has been accomplished with difficulty, by prolonged treatment of *p*-cymene, or 2,6-dinitrocymene, either with a mixture of fuming nitric acid and concentrated sulphuric acid (9, 12), or with a mixture of fuming nitric acid and oleum (13). The principal product was probably 2,3,6-trinitrocymene and was obtained probably in low yield.

The removal of alkyl groups attached to aromatic nuclei (dealkylation) under nitrating conditions has been repeatedly observed by a number of

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Contribution from the Division of Organic Chemistry, McGill University, Montreal, Que.

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Que.

workers (Claus (7), Qvist (24), Smith *et al.* (25, 26, 27, 28)). The removal of an isopropyl group during the nitration of a *p*-cymene derivative (*p*-cymene-*o*-sulphonic acid) was first observed by Hintikka (14). The same process was probably responsible for the appearance of a toluene derivative on the nitration of 2-chlorocymene as observed by Lubs and Young (20). Doumani and Kobe (8) have given detailed consideration to dealkylation as it accompanies mononitration of *p*-cymene. The loss of an isopropyl group was also observed by Alfthan (1) during the dinitration of *p*-cymene, and by Halse and Dedichen (13) during the trinitration of cymene.

Several workers have speculated about the nature of the dealkylation process. Claus and Qvist (7, 24) have suggested that the first step is oxidation of the alkyl group to a carboxyl group by nitric acid, followed by substitution of the carboxyl group by a nitro group. Smith *et al.* (25, 26, 27, 28) have postulated that methylbenzenes are first converted to benzyl nitrates by nitric acid, and these in turn to nitro compounds by sulphuric acid. Doumani and Kobe (8) have criticized these views, stating that oxidation plays no part in the process, for if the *p*-cymene is properly emulsified during nitration no oxides of nitrogen are formed. Rather they suggest that the *p*-cymene is cleaved directly by the nitric acid, the tolyl group appearing as a nitrotoluene and the isopropyl group as propanol-2. They were able to isolate propanol-2 from the spent mixed acid, though admittedly not in quantitative yields.

The present investigation was undertaken to determine optimum conditions for the preparation of 2,6-dinitrocymene and 2,4-dinitrotoluene and to devise improved methods for the separation of these compounds.

Experimental Section

Purification of p-Cymene

Sulphite turpentine (secured from the Brown Corporation, Berlin, N.H.) had been purified by steam distillation from sodium hydroxide solution, and was subsequently treated with cold 95% sulphuric acid until the acid was no longer colored, washed with dilute sodium bicarbonate solution, dried over calcium chloride, and distilled through a five-plate column. *p*-Cymene, having the following physical constants, boiling range (corr.) 176.8° to 177.3° C., n_D^{20} 1.4906 to 1.4909, was used in all the runs reported below. By actual experiment it was found that a less pure grade of *p*-cymene, obtained by washing with alkali followed by steam distillation, give substantially the same results on nitration. In agreement with Mann *et al.* (21) it has been found that refluxing over sodium metal does not yield a pure product.

Standard Conditions of Nitration

A convenient nitration vessel was found to be a 500 cc., three-necked, round-bottomed Pyrex flask, equipped with standard taper ground glass joints and surrounded by a cooling bath adjusted to a temperature about 5° C. below the designated reaction temperature. The flask was equipped with a

separatory funnel and carbon dioxide inlet tube, an efficient stirrer with plain bearing, and a thermometer with bulb immersed in the reaction mixture. All nitrations were carried out under carbon dioxide and with very rapid stirring. The separatory funnel was calibrated and its outlet tube drawn down to a fine jet to assist in obtaining a uniform rate of delivery.

A solution of 60 gm. (0.915 mole) 96% nitric acid and 120 gm. (1.175 moles) 96% sulphuric acid was placed in the nitration vessel and the temperature adjusted to 30° C. by external cooling. To this was added 30 gm. (0.224 mole) *p*-cymene with vigorous stirring over a period of two hours \pm 10 min. Stirring was continued for an additional hour. Throughout the addition and stirring periods the temperature of the reaction mixture was maintained to within one degree of that desired. The reaction mixture was then poured onto ice.

The water layer was separated and extracted repeatedly with benzene; the washings were then combined with the nitration product proper. The benzene solution was washed several times with water containing a little salt and with 10% sodium carbonate solution. Separation of the benzene layer was greatly assisted by filtering to remove a small quantity of suspended solid material. The organic layer was then dried over anhydrous sodium sulphate, and the benzene removed at reduced pressure (100 mm.).

Identification of the Nitration Products

A partial separation of the nitration product into its components was achieved by fractional distillation under reduced pressure. Because of the highly viscous nature of the material and the close similarity of the boiling point curves of 2,4-dinitrotoluene and 2,6-dinitrocymene, it was impossible to do more than separate the mono from the dinitro derivatives. Fractionation was carried out in a $\frac{1}{2}$ by 12 in. jacketed column loosely packed with three-turn $\frac{3}{8}$ in. diam. helices. Unreacted *p*-cymene was taken off as a forerun at 100 mm. pressure, the distillation proper being carried out at 2.5 to 4.0 mm. The first fraction (generally small) consisted mainly of 2-nitrocymene and a little 4-nitrotoluene. Fraction II contained the 2,6-dinitrocymene and 2,4-dinitrotoluene. High boiling residue left in the still pot was small in amount.

The determination of the proportions of 2,4-dinitrotoluene and 2,6-dinitrocymene in Fraction II was accomplished by measuring the refractive index at $40 \pm 0.2^\circ$ C. The composition could then be determined from Table I.

An investigation into the possibility of separating 2,6-dinitrocymene from 2,4-dinitrotoluene in Fraction II by fractional crystallization was also undertaken. Alfthan (1) had suggested ethanol and Kyker and Bost (18) had suggested methanol as suitable solvents. These were not found to be satisfactory. However, good results were obtained with Skelly Solve B (b.p. 60° to 68° C.). Fraction II was shaken with three times its weight of this solvent at room temperature. After settling, the solvent was decanted and cooled to 0° C. The oil that separated was removed and the supernatant

liquid again cooled to -60°C . (or the solvent simply evaporated off). The solid that separated was very rich in 2,6-dinitrocymene. By actual test 2,6-dinitrocymene containing 30% dinitrotoluene by weight after such a

TABLE I
REFRACTIVE INDEX - COMPOSITION VALUES FOR
VARIOUS DINITROTOLUENE-DINITROCymENE
MIXTURES

Per cent dinitrotoluene by weight	n_D^{40}
0	1.5368
8.97	1.5400
17.20	1.5428
22.75	1.5447
28.10	1.5468
35.15	1.5491
50.00	1.5543
60.35	1.5580
M.p. dinitrocymene	53° to 54°C .
M.p. dinitrotoluene	71° to 72°C .

treatment gave an oil in 13% yield from the first crystallization at 0°C . and a solid in 23% yield from the second stage. The oil contained 19% dinitrotoluene and the solid 6% dinitrotoluene. Substantially pure 2,6-dinitrocymene was obtained by seeding an alcohol solution of this solid. By a repetition of this process the entire nitration product could be separated. This method of purification proved to be too time consuming, however, to be used as routine method of analysis for the large number of runs involved.

Experimental Results

Each of the various variables (temperature, nitric acid concentration, sulphuric acid concentration, etc.) was investigated over a suitable range,

TABLE II
EFFECT OF VARIATION OF TEMPERATURE

Run No.	Temp., $^{\circ}\text{C}$.	Weight of products, gm.		Residues, gm.	D.N.T. in mixture, %	Yield, %	
		Mononitro	Dinitro			D.N.C.	D.N.T.
1 & 2*	-20	—	37.3	4.7	28.2	53.3	25.9
3	-6	—	38.8	5.0	27.5	56.0	26.1
4	6	0.1	39.0	2.6	27.0	56.6	25.8
5	16	—	37.9	3.9	27.0	55.2	25.1
6 & 7*	30	1.1	34.6	5.0	27.1	50.3	23.0
8	40	2.2	30.3	5.3	27.7	43.6	20.6
9	60	1.6	21.5	6.0	33.4	28.5	17.6

* Average of two runs.

the remaining variables being maintained at the values indicated under "standard nitrating conditions". The results obtained have been summarized in Tables II to VII with an accompanying graph in the case of Table IV.

TABLE III
EFFECT OF VARIATION OF NITRIC ACID CONTENT OF MIXED ACIDS

Run No.	96% HNO ₃ , gm.	Weight of products, gm.		Residues, gm.	D.N.T. in mixture, %	Yield, %	
		Mononitro	Dinitro			D.N.C.	D.N.T.
25*	24.5	0.3	13.0	4.0	28.5	18.5	9.1
26	41.2	2.3	24.2	3.0	27.8	34.8	16.5
28	48.7	3.4	28.6	3.7	27.5	41.3	19.3
6	60.0	1.1	34.6	5.0	27.1	50.3	23.0
31	67.5	0.2	35.3	5.0	26.2	51.9	22.7
32	70.0	—	37.0	4.9	26.5	54.2	24.1
33	100.0	0.1	37.8	4.3	28.2	54.0	26.2

* Difficult to prevent "fume-off". Considerable quantities (15 gm.) of unchanged *p*-cymene recovered.

TABLE IV
EFFECT OF VARIATION OF SULPHURIC ACID CONTENT OF MIXED ACIDS

Run No.	96% H ₂ SO ₄ , gm.	Weight of products, gm.		Residues, gm.	D.N.T. in mixture, %	Yield, %	
		Mononitro	Dinitro			D.N.C.	D.N.T.
17*	0	7.9	9.7	5.6	32.7	12.9	7.8
18	40	8.5	18.0	5.5	29.5	25.3	13.0
19	70	7.1	25.9	5.4	28.6	36.8	18.2
20	100	4.3	31.2	4.3	27.7	44.9	21.2
6	120	1.1	34.6	5.0	27.1	50.3	23.0
22	150	0.3	36.6	4.1	27.7	52.7	24.9
23	200	0.3	37.1	4.1	27.6	53.5	25.1

* In this run, 2.9 gm. of unchanged cymene recovered.

TABLE V
EFFECT OF VARIATION OF ADDITION TIME

Run No.	Addition time, hr.	Weight of products, gm.		Residues, gm.	D.N.T. in mixture, %	Yield, %	
		Mononitro	Dinitro			D.N.C.	D.N.T.
38	1/3	—	38.5	3.6	25.8	56.8	24.3
39	1	0.1	37.7	3.5	26.3	55.3	24.3
41	2	1.2	35.9	4.1	26.7	52.4	23.4
42	5	2.1	33.4	4.1	27.6	48.2	22.6
43	8	2.6	31.0	5.3	29.5	43.5	22.4
44	11	2.1	30.0	5.6	29.4	42.2	21.6

In all cases "Weight of products" excludes unreacted *p*-cymene, if any, and alkali-soluble or alkali- and benzene-insoluble products, if any. "Residues" means the weight of nondistillable material. "D.N.T. in mixture" means

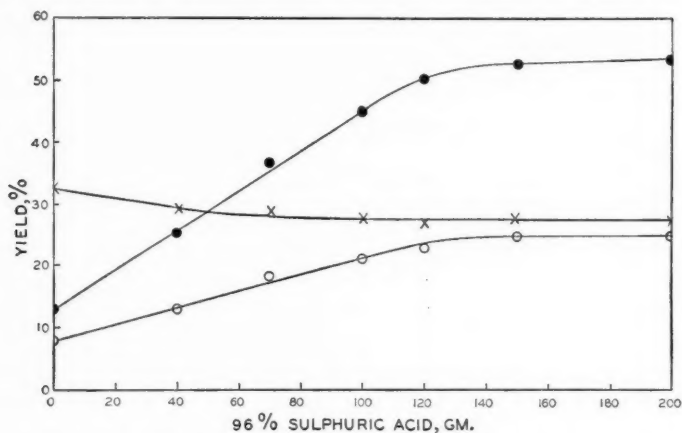


FIG. 1. Effect of variation of sulphuric acid content on nitration of *p*-cymene.

- Yield of dinitrocymene.
- Yield of dinitrotoluene.
- × Per cent dinitrotoluene in mixture.

TABLE VI

EFFECT OF VARIATION OF STIRRING TIME AFTER COMPLETE ADDITION OF CYMENE

Run No.	Stirring time, hr.	Weight of products, gm.		Residues, gm.	D.N.T. in mixture %	Yield, %	
		Mononitro	Dinitro			D.N.C.	D.N.T.
34	1/2	1.4	33.9	4.4	26.6	49.6	22.2
35	1	1.1	34.6	4.3	27.1	50.2	23.0
36	2	1.2	35.9	4.2	26.4	50.8	22.5
37	3	0.8	35.6	—	26.7	52.0	23.4

the per cent dinitrotoluene in the dinitration fraction. "Yields of dinitrocymene and dinitrotoluene" were calculated on the total amount of *p*-cymene used; no allowance was made for *p*-cymene recovered.

With the object of comparing the relative ease of the nitration of cymene with the nitration of other simple aromatic compounds, the mononitration of cymene was attempted by means of a typical procedure employed in the mononitration of benzene. A nitrating mixture consisting of 25.5 gm. 96% sulphuric acid and 20.1 gm. 70% nitric acid was added dropwise over a period of 70 min. to 30 gm. of cymene at 30° C. with vigorous stirring. The mixture was then heated to 56° C. and maintained at this temperature for 50 min.,

TABLE VII
 MISCELLANEOUS VARIATIONS IN NITRATING CONDITIONS

Run No.	Weight of products, gm.		Residues, gm.	D.N.T. in mixture, %	Yield, %	
	Mononitro	Dinitro			D.N.C.	D.N.T.
46	1.5	35.3	3.9	27.2	51.0	23.5
47	1.4	34.8	4.4	27.0	50.6	23.0
48	1.6	34.1	5.0	27.2	49.5	22.8
51	1.2	35.5	4.7	27.7	51.1	24.2
54	—	2.3	3.3	20.4	3.6	1.2
55	—	20.5	9.9	29.4	28.9	14.8
59	11.7	11.9	2.7	25.5	19.0	7.7
60	0.8	27.4	3.7	19.0	59.0	17.1
64	—	38.7	3.8	26.8	56.4	25.4

NOTES:

- Run 46 Standard nitrating conditions. Small amount of cobalt nitrate present.
 Run 47 Standard nitrating conditions. Small amount of zinc nitrate present.
 Run 48 Standard nitrating conditions. Small amount of nickel nitrate present.
 Run 51 Standard nitrating conditions. Nitric acid decolorized with urea nitrate.
 Run 54 Standard nitrating conditions. Nitric acid added to cymene in sulphuric acid.
 Run 55 Standard nitrating conditions. Mixed acids added to cymene.
 Run 59 Nitration of 2-nitrocymene under conditions employed in adding second nitro group to mononitrotoluene (see text).
 Run 60 Nitration of mononitrocymene under standard nitrating conditions using one-half the mixed acid normally employed.
 Run 64 Nitration done under optimum conditions for all variables (see Discussion of Results).

with continuous stirring. The now nearly black solution was poured onto ice and worked up in the usual manner. A forerun of 13.6 gm. of unchanged cymene was followed by 5.3 gm. of yellow product boiling over a wide range (65° to 122° C. at 14.8 mm.) though the main fraction came off at temperatures corresponding to mononitrated material. A considerable amount of residue (9.3 gm.) remained in the pot.

The ease of addition of a second nitro group to mononitrocymene under conditions that are known to operate successfully in a similar nitration stage in the production of T.N.T. was also determined. To an acid mixture composed of 15.6 gm. of 96% nitric acid, 42.4 gm. 96% sulphuric acid, and 4.95 gm. of water at 85° C., 30 gm. of mononitrocymene ($n_D^{20} = 1.4291$) was added over a 20 min. period with stirring. After being held at 92° C. for 10 min., the mixture was allowed to stand for a similar length of time before pouring on ice. The results are tabulated in Table VII, Run 59.

Discussion of Results

Effect of Temperature (Table II)

Maximum yields of dinitrocymene and dinitrotoluene were obtained at approximately 6° C. This is in contradiction to the results of Kyker and Bost (18), who reported that the absolute yields steadily increase with fall

in temperature. Production of nitrogen oxide fumes during nitration increased with rising temperature until at 60° C. oxidation was so violent that mixtures tended to fume off. The color of the undistilled products also became progressively darker (they were almost black at 60° C.). The quantity of alkali-soluble materials produced during nitration increased in the same way, a very marked rise taking place at 60° C. Hence, lowered yields at higher temperatures are probably due to the occurrence of oxidation and sulphonation as side reactions.

Quantities of alkali- and benzene-insoluble material were produced in increasing amounts as the temperature was reduced below 30° C. The nature of this product was not determined, but its appearance probably accounts for the drop in yield of nitrated products at temperatures below the optimum.

Effect of Nitric Acid Content of the Nitrating Mixture (Table III)

The effect of variations in the nitric acid content of the nitrating mixture upon the ratio of the dinitrotoluene to dinitrocymene in the products appears to be negligible. (There is a suggestion of a minimum at 70 to 75 gm. nitric acid for 30 gm. *p*-cymene). When the quantity of nitric acid was below 70 gm., the yield of dinitrocymene and dinitrotoluene dropped rapidly, however. Under these conditions oxidation was extensive, large volumes of nitrogen oxide fumes were produced and the quantity of alkali-soluble products formed increased rapidly. Considerable amounts of cymene were also recovered unchanged. The yield of dinitrated products remained substantially unaltered when the nitric acid used was increased beyond 70 gm. This is the amount required assuming quantitative dinitration of the cymene and allowing sufficient acid to convert the isopropyl groups removed (as the result of dinitrotoluene formation) into isopropyl nitrate.

Effect of Sulphuric Acid Content of the Nitrating Mixture (Table IV)

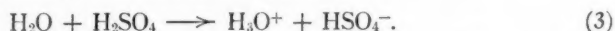
It has been shown by Bennett *et al.* (5) that the vigorous nitrating action of mixed acids is due to the presence of the active nitronium ion, NO_2^+ , formed by the reaction:



Nitration of an aromatic compound is stated to be due to simultaneous attack on the aromatic nucleus by the nitronium ion and a proton acceptor such as HSO_4^- :



In such acid mixtures sulphuric acid is immobilized by an amount equivalent to the water present since there is no doubt that water reacts in an excess of sulphuric acid completely according to the equation:



Hence it is only the sulphuric acid in molar excess of this water that is effective in the production of the active nitrating agent by Equation (1). Nitration requires not only the attachment of a cation to the benzene nucleus, however,

but also the removal of a proton. The most obvious proton acceptor in a water-sulphuric acid medium is the bisulphate ion, and its concentration is increased with increase in water content. Increase in the water content, however, reduces the concentration of NO_2^+ ion, hence optimum nitrating condition will be a compromise between these two opposing factors. Bennett *et al.* (6) have shown that 92% sulphuric acid is the medium in which the velocity of nitration of 2,4-dinitrotoluene is a maximum. Martinsen (22) found that the velocity of nitration of a number of substituted benzenes was greatest in 90% sulphuric acid. Hence the most favorable concentration appears to be approximately 90 to 92%.

Using 30 gm. (0.224 mole) cymene and 60 gm. (0.915 mole) 96% nitric acid, experimental results obtained in this investigation have shown that the yield of both dinitration products increases with the amount of sulphuric acid present, reaching a maximum at approximately 130 gm. The water content of a typical run carried out with this amount of acid was determined at the beginning and end of the nitration with the Karl Fisher reagent. A mean of these two figures can be taken as the average water content of the system during the reaction. The value obtained, 93%, is comparable with Bennett's figure of 92%. Beyond this point, further amounts of sulphuric acid do not appear to have any marked influence, as can be seen by Fig. 1.

The ratio of dinitrotoluene to dinitrocymene remained practically constant at about 27.5% when the sulphuric acid content of the nitrating mixture was 100 gm. or more for 30 gm. *p*-cymene. When the amount of acid was decreased below this amount a slight rise in this ratio occurred. Increased oxidation was observed at the lower sulphuric acid contents. Preferential attack on the isopropyl group in the cymene during this side reaction could account for such a rise in the dinitrotoluene-dinitrocymene ratio.

Effect of Variation of Addition Time (Table V)

Over-all yields of dinitrated products dropped gradually with increase in the addition time of the cymene to the nitrating mixture. A corresponding increase in the quantity of oxidation products occurred; this probably accounts for lowered yields and for a rise in the dinitrotoluene-dinitrocymene ratio.

Effect of Variation of Stirring Time (Table VI)

This variable does not appear to have an appreciable effect on the products obtained.

Miscellaneous Variations in Nitrating Conditions (Table VII)

Very poor results were obtained when the mode of addition of the reactants was other than that stipulated under "standard nitrating conditions". In Run 54, the nitric acid was added dropwise to the mixture of cymene and sulphuric acid. Considerably more heat was evolved than in a normal run and the mixture had a great tendency to fume off. Since the amount of dinitrated material was extremely small, the value for "D.N.T. in mixture" (20.4%) means little. In Run 55, the combined mixed acids were added to

the cymene, with results similar to those of Run 54 though yields were higher. The behavior of cymene in this respect is in contrast with that of other aromatics such as benzene and toluene.

The addition of various metallic nitrates (Runs 46, 47, 48) appeared to have no appreciable effect on the results. Phillips (23) reported that in the sulphonation of cymene, the addition of small quantities of such metallic salts also had little effect.

It will be observed that on mononitrating 2-nitrocymene by the "standard conditions" (making suitable corrections in the quantities of nitrating mixture employed—Run 60), the amount of dinitrotoluene produced dropped from the normal 27% to a value of 19%. The difference in these percentages (i.e., 8%) is the value for the amount of *p*-nitrotoluene produced in the mononitration of cymene as found by Kobe and Doumani (15). It would thus appear that dinitration of cymene proceeds in two stages. In the mononitration stage the per cent cleavage of the isopropyl group is 8%; in the next stage a further 19% is removed. The greater cleavage that occurs in the second stage would be anticipated since the nitro group is *meta* directing—hence it aids removal of the isopropyl group which in 2-nitrocymene is *meta* to it.

From an analysis of the various tables it is possible to select conditions that should give maximum yields of products. These conditions are as follows (for a 30 gm. sample of cymene): 96% nitric acid, 75 gm.; 96% sulphuric acid, 150 gm.; temperature, about 0° C.; addition time, as short as possible; stirring time, not critical but conveniently one hour. The results of a nitration carried out under these conditions will be found in Table VII—Run 64 (an addition time of 15 min. being used.) The yields were not appreciably better than those obtained under the conditions of optimum addition time alone (Table V—Run 38). It would appear, then, that there is but little possibility of increasing these values beyond this point.

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FURTHER DEVELOPMENTS IN THE VISTEX METHOD FOR THE DETERMINATION OF THE INTRINSIC VISCOSITY OF HIGH POLYMERS¹

BY D. A. HENDERSON AND N. R. LEGGE

Abstract

The intrinsic "vistex" viscosities of several series of butadiene-styrene copolymers of varying conversion and average molecular weight, dissolved directly from the latex in the vistex solvent mixture (toluene-isopropanol, 80/20 by volume), have been investigated and compared with the intrinsic viscosities of the corresponding coagulated, dried polymers dissolved in toluene. The intrinsic viscosity in toluene, $[\eta]_T$, is related to the intrinsic vistex viscosity, $[\eta]_V$, in toluene-isopropanol by the equation:—

$$\log[\eta]_T = 0.0827 + 1.08 \log [\eta]_V.$$

Hence, viscosity average molecular weight may be calculated from vistex measurements.

A further development of the method has shown that, once the latex is dissolved in the vistex solvent, the solution may be diluted, within certain defined limits, by the addition of pure solvent (toluene) to obtain the several levels of concentration of polymer required for the determination of intrinsic viscosity. It is then possible, by extrapolation to zero concentration of polymer, to obtain a value for the intrinsic viscosity that is equal to the conventional intrinsic viscosity of the polymer in pure solvent after coagulation and drying under very mild conditions. The viscosity characteristics of butadiene-styrene copolymers of varying conversion appear to be represented, at conversions below the gel point, by the equation

$$\frac{\ln \eta_r}{c} = [\eta] - \beta'[\eta]^nC,$$

where β' and n are constants of the order of 0.25 and 1 for solutions in toluene and 0.1 and 2.5 respectively for vistex solutions. Distinct changes in β and/or n have been found at conversions in the region of and beyond the gel point.

Introduction

The term vistex was first used by Baker *et al.* (5) in 1943 to describe measurements of the dilute solution viscosity of polymer dissolved directly from the latex in a mixture of hydrophobic and hydrophilic solvents such as xylene and pyridine. Thus, the introduction of further unknown variables by coagulation and drying was avoided and a more direct evaluation of polymerization variables could be obtained. The method was primarily developed to follow changes in polymer characteristics with conversion during the production of GR-S.

The type and amount of hydrophilic solvent required to bring the water into solution was found to be fairly critical. In agreement with the results

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Contribution from the Physical Research Laboratory, Research and Development Division, Polymer Corporation, Sarnia, Ont.

with other systems (1) it was found that the addition of the hydrophilic non-solvent lowered the inherent viscosity* of the polymer. A number of solvent systems were tried and preference was given to systems in which the highest relative viscosity was obtained, on the grounds that the most highly extended chains would give the highest viscosity increment per unit chain length. The solvent system chosen was xylene-pyridine (75/25) which was found to give clear solutions of 1 ml. of GR-S latex per 100 ml. of solution at 40° C. Viscosity measurements had to be made at that temperature.

It was known that the inherent viscosity of the polymer increased at higher conversions and Baker *et al.* (5) postulated that there should be a maximum in the inherent vistex viscosity vs. conversion relation if measurements are made up to and beyond the gel point. Although the presence of microgel of high swelling volume may raise the inherent viscosity somewhat, as the density of cross linkages increases this contribution of dispersed microgel particles to the inherent viscosity will decrease and the inherent viscosity will drop. This maximum in the curve was found (5, 6, 14) to occur at the same conversion for both vistex and intrinsic viscosity data.

Later, Kolthoff (15) proposed the use of benzene-isopropanol (80/20) as a vistex solvent. This system dissolved the latex immediately without heating and permitted the determination of viscosity at room temperature. Inherent viscosities obtained with this system were somewhat lower than those determined in the xylene-pyridine system but gave relatively the same information. The addition of aqueous alkali or soap to the vistex system caused a reduction in the inherent viscosity that was due only to the amount of water added. Generally, nonsolvent was found to lower the inherent viscosities of polymers of high molecular weight much more than those of polymers of low molecular weight. Harris and Kolthoff (12) correlated values obtained by the vistex method (benzene-isopropanol) with inherent viscosities in benzene for a number of GR-S type polymers. The inherent viscosity in benzene, $\{\eta\}_B$, was found to be related to the inherent vistex viscosity, $\{\eta\}_{BIW}$, by the following equation:—

$$\{\eta\}_B = A \cdot \{\eta\}_{BIW},$$

where A varies from 1.16 at $\{\eta\}_{BIW} \simeq 0.3$ to 1.42 at $\{\eta\}_{BIW} \simeq 1.8$. It was emphasized that the table of correlated data was valid only if 1 gm. of partially vented latex was used per 100 ml. of solution.

* Terminology used is in accordance with that proposed by Cragg (7) except that we have introduced the word "vistex" into the appropriate terms when the vistex method was used, i.e., the polymer dissolved directly from the latex. Brief definitions are given below:—

ν_{solution} = kinematic viscosity of the polymer solution,

ν_{solvent} = kinematic viscosity of the solvent,

$$\nu_r = \frac{\nu_{\text{solution}}}{\nu_{\text{solvent}}},$$

$$\frac{\ln \nu_r}{c} = \text{inherent kinematic viscosity,}$$

$$\lim_{c \rightarrow 0} \frac{\ln \nu_r}{c} = [\nu] = \text{intrinsic kinematic viscosity.}$$

It was noted in reviewing all the previous work that inherent viscosity, $\frac{\ln \eta_r}{c}$, rather than its limiting value, the intrinsic viscosity, $\lim_{c \rightarrow 0} \frac{\ln \eta_r}{c}$, was determined. This is common practice in rapid, routine determinations. However, these one-point determinations and also those suggested by Cragg *et al.* (9) have been found inadequate to represent the viscosity characteristics of the wide ranges of polymers studied in our laboratories. Moreover, there did not appear to be available any suitable method of calculation of the intrinsic viscosity of the polymer in a good solvent and hence the viscosity average molecular weight, from the corresponding intrinsic viscosity. Therefore, a further study of the viscometer method was commenced. The solvent system chosen was toluene-isopropanol (80/20) and limiting, intrinsic values were determined throughout the investigation. Intrinsic viscosities of several series of butadiene-styrene copolymers of varying average molecular weight and conversion have been determined and correlated with the intrinsic viscosities of the coagulated, dried polymer in toluene and also in the viscometer solvent.

Experimental Procedure

Viscometer Viscosity Procedure

The latex, about 1 ml., was added to about 100 ml. of the solvent mixture and dissolved readily after a few minutes agitation. The latex can be weighed precisely and the concentration calculated from its solids content. However, it was frequently found advisable to filter the solution through a sintered glass filter, porosity C, and this often caused a change in concentration. The concentration of the solution was determined by evaporating 20 ml. on a steam plate for about 15 min. after the solvent had disappeared. The concentration value was corrected for nonpolymer content and expressed in grams of polymer per 100 ml. of solution.

The solutions were brought to $30.4 \pm 0.03^\circ \text{C.}$ by suspending the flask in the bath for at least 10 min. The flow times were measured in an Ubbelohde viscometer modified by the installation of a large bulb to allow dilution of the solution while in the viscometer.

The viscometer had the following characteristics:—

L , length of capillary	8.97 cm.
R , radius of capillary (precision bore)	0.254 cm.
H , mean hydrostatic head (head at $t/2$ sec.)	12.12 cm.
t_0 , time of efflux at 30.4°C. for toluene	105.0 sec.
for toluene-isopropanol (80/20)	112.2 sec.
V , volume of efflux	= 4.03 ml.
Lt_0/V for toluene	= 233.8
τ , mean shearing stress at the wall ($Hdg R/2L$)	= 14.4

Kinetic energy corrections were determined with water and toluene in the necessary temperature range using the equation

$$v = At + B/t. \quad (1)$$

These corrections have been applied to all measurements. As pointed out by Rogers *et al.* (17) the error introduced by using the intrinsic kinematic viscosity, $[\nu]$, for the intrinsic viscosity, $[\eta]$, is negligible.

Exactly 20 ml. of solution was pipetted into the viscometer and measurements of flow time were made in duplicate or triplicate until agreements within ± 0.1 sec. were obtained. Then the solution in the viscometer was diluted by the addition of 10 ml. of solvent mixture and the flow time again measured after thorough mixing. The same procedure was repeated with further additions of 10 ml. and 20 ml. of solvent mixture. Concentrations are $\frac{2}{3}$, $\frac{1}{2}$, and $\frac{1}{3}$ of the original value.

With each dilution, the flow time of the solvent was changed because of the change in the concentration of water (considering it as part of the solvent mixture). From the concentration of the solution and the latex solids, it is possible to calculate the percentage of water present in the solution. Or, alternatively, it may be determined by a Karl Fischer titration (both methods have been used by us and found to agree satisfactorily). The values of the solvent flow time corresponding to each dilution were read from a graph relating flow time to percentage composition of the solvent. After each run the viscometer was cleaned by flushing with toluene and dried carefully. It was cleaned periodically by standing overnight in concentrated nitric acid.

Solutions of the coagulated polymers were prepared by allowing about 0.25 gm. to stand overnight in toluene. They were freed of extraneous material by filtering through a sintered glass filter, porosity C, and the concentration determined in a manner similar to the vistex solutions. The viscosity measurements were made as described above. The nonpolymer corrections were assumed to be similar to those used in the vistex method. This appears to be true for GR-S, within the experimental error.

The solvents used in these measurements were both reagent grade, the toluene supplied by the Nichols Chemical Company, Toronto, Ontario, and the isopropanol by the Shell Oil Company, London, Ontario.

Preparation of Latices

Butadiene-styrene copolymers of varying average molecular weight and conversion were prepared in the Polymerization Research Laboratory under the direction of Dr. H. L. Williams.

Formula:

Butadiene (98%)	71.50 parts
Styrene	28.50 "
DDM	Variable
Water	180 parts
Emulsifier (RRC flakes—100%)	2.15 "
Potassium persulphate	0.23 "
Sodium hydroxide	to pH 10.0 - 10.5
Polymerization temperature	50° C.

The reactions were stopped with 0.16 part of hydroquinone (dissolved in water) per 100 parts of monomer. Conversions were determined on the unvented latex using a syringe, and the latices were stabilized with BLE* (1.5 parts per 100 parts by weight of dry rubber, calculated on the basis of conversion) and stripped at atmospheric pressure with live low pressure steam until there appeared to be no styrene left. The time required for stripping varied from 30 to 60 min., the actual time depending upon the conversion. During stripping, the latex reached a temperature of about 80° C. One-half of the stabilized and stripped latex was coagulated (12% brine - 0.1% sulphuric acid) and dried in an air oven. The low conversion polymers required periods of several hours drying at 40° to 50° C. High conversion samples were dried for 30 min. at 80° C.

Experimental Results and Discussion

Intrinsic Vistex Viscosity

In the usual determination of intrinsic viscosity, the solvent system remains constant in composition as the system is diluted to obtain the levels of polymer concentration necessary for extrapolation to the limiting value. However, in the determination of intrinsic vistex viscosity, where the percentage of the nonsolvent water in the latex is usually variable, the maintenance of a constant composition of solvent throughout the dilution is often difficult and did not appear to be consistent with the requirement for a rapid, precise determination. Therefore, dilution with the vistex solvent alone was attempted and the effect of variation of the percentage of water in the latex on the intrinsic vistex viscosity was examined.

Standard plant GR-S latex was added to the vistex solvent in the usual manner, then different amounts of water were added to produce a variation in the water content. At three levels of water, the vistex determination was run in the usual manner, diluting with toluene-isopropanol. Flow times for the solvent system were determined by interpolation on a plot of flow time vs. water content of a toluene-isopropanol-water (80/20/variable) system.

The intrinsic vistex viscosity values, shown in Fig. 1, were found to be independent† of the water content of the latex, within the limits where clear solutions were obtained, but the slope of the inherent vistex viscosity vs. concentration line was directly proportional to the percentage of water in the latex. This is in agreement with Tingey *et al.* (19)—this reference was discovered in a later search of the literature—who found that fractions of GR-S in benzene containing a small percentage of methanol could be diluted with pure benzene during the viscosity determination to give the true limiting value of the viscosity in pure benzene.

In further experiments, raw polymer was dissolved in the vistex solvent and the intrinsic viscosity determined, then about 1% of water was added

* BLE—an antioxidant, Naugatuck Chemical Co.

† It should be noted that the intrinsic vistex viscosity is not independent of the percentage of water in the vistex solvent (about 0.06%) that is used for dilution.

to the original vistex solution and the determination repeated, diluting with vistex solvent as above. This procedure was repeated at a number of levels of water. All the intrinsic viscosity values were identical with the first and the change of slope was similar to that shown in Fig. 1.

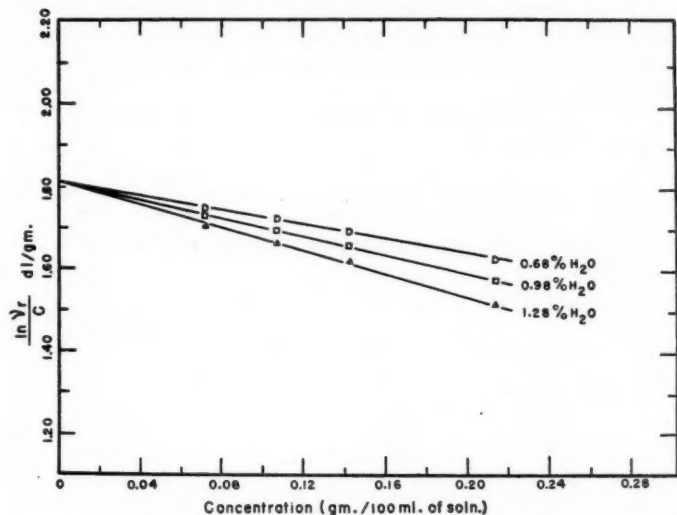


FIG. 1. Effect of amount of water in vistex solution on vistex measurements.

The basis for the method of determination of intrinsic vistex viscosity was thus established. The relation between the percentage of water and the slope of the inherent vistex viscosity vs. concentration line was used in the correction of slope data to be discussed later.

Correlation of Intrinsic Vistex Viscosity and Intrinsic Viscosity in Toluene

Several series of butadiene-styrene copolymers of varying average molecular weight* and conversion were prepared for investigation of the vistex method.

Preliminary work with the vistex method indicated normal behavior to be expected from a solution of polymer in the presence of nonsolvent.** Therefore, it was postulated that there should be a correlation between the intrinsic viscosity of the coagulated, dried polymer in toluene and the intrinsic vistex viscosity determined on the corresponding latex, providing further variables are not introduced during coagulation and drying. When this correlation is established the intrinsic vistex viscosity values may then be used to calculate viscosity average molecular weights.

* The average molecular weight was varied by the amount of modifier, dodecyl mercaptan, DDM, added. The larger the amount of mercaptan added the lower is the average molecular weight. The polymerization formula for standard GR-S contains 0.42 part of DDM.

** The literature has indicated adequate correlations of the intrinsic viscosity of polymer in a good solvent with that in a poor solvent.

As a basis for this correlation and to permit an evaluation of the variables of coagulation and drying, the intrinsic viscosities of coagulated, dried polymer in toluene were correlated with the corresponding values in toluene-isopropanol (80/20).

Intrinsic viscosities in toluene $[\nu]_T$, and in the vistex solvent, $[\nu]_{VS}$, for three series of experimental polymers are shown in Table I. When plotted on a logarithmic scale in Fig. 2 these data give a linear relation represented by the equation:—

$$\log [\nu]_T = 0.0827 + 1.08 \log [\nu]_{VS}. \quad (2)$$

TABLE I

INTRINSIC VISTEX VISCOSITIES AND CORRESPONDING INTRINSIC VISCOSITIES OF COAGULATED, DRIED POLYMER IN TOLUENE AND VISTEX SOLVENT. BUTADIENE-STYRENE COPOLYMER

No.	Parts DDM	Conv., %	Intrinsic vistex	Intrinsic viscosity	
				In toluene	In vistex solvent
MM—11,3,42					
3	0.40	55.1	—	1.68	1.35
4	0.45	56.0	—	1.45	1.19
5	0.50	54.2	—	1.33	1.08
6	0.60	55.3	—	1.10	0.94
MM—48,1,28—48					
1	0.32	23.0	1.12	1.47	1.21
2	"	27.2	1.19	1.55	1.26
3	"	40.3	1.45	1.86	1.42
4	"	48.0	1.58	2.01	1.60
5	"	57.6	1.69	2.13	1.72
6	"	65.3	1.94	2.58	2.03
7	"	75.0	2.21	2.97	2.29
8	"	82.8	1.90	2.57	2.01
MM—48,4,6—54					
1	0.42	18.6	1.08	1.34	1.11
2	"	25.6	1.27	1.65	1.30
3	"	42.4	1.52	1.92	1.51
4	"	57.8	1.64	2.01	1.58
5	"	67.8	1.91	2.46	2.03
6	"	73.0	1.89	2.40	1.95
7	"	76.8	2.11	2.64	2.15
8	"	81.9	2.00	2.76	2.15
X-387				2.36	1.82
Plant runs				2.37	1.88

Values for standard GR-S were found to fit this relation. A further confirmation was found in the work of Cragg and Rogers (8) who report the intrinsic viscosity of Sarnia GR-S to be 2.07 in benzene and 1.60 in benzene-isopropanol (80/20), compared with 1.64 for the latter value calculated from their $[\eta]_T = 2.07$ and Equation (2). The divergence between Cragg's value and our value of $[\nu]_T$ for GR-S is due mainly to the fact that our values are corrected for nonrubber material and to some difference in calibration methods.

A relation very similar to Equation (2) is reported by Mark *et al.* (11) for the intrinsic viscosities of polystyrene in toluene and method ethyl ketone. A review and recalculation of the data of Harris and Kolthoff (12, 14) has shown

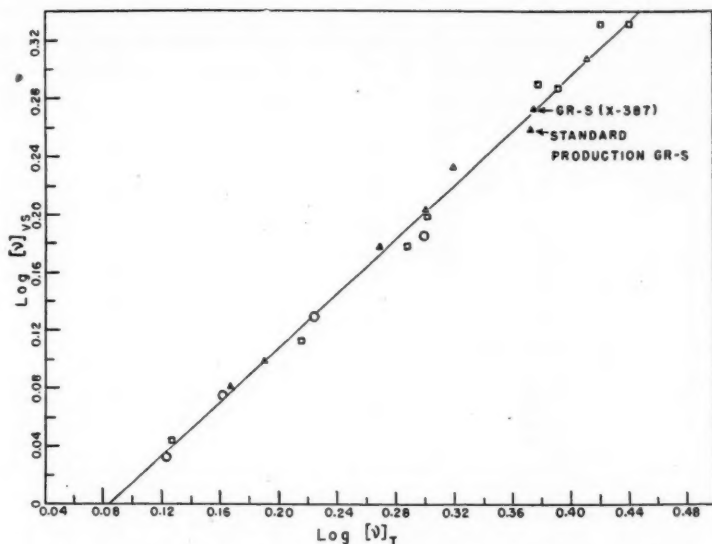


FIG. 2. Correlation between viscosity of polymer in toluene, $[\nu]_T$, and in vistex solvent, $[\nu]_{VS}$.

- charge MM-11, 3, 42
- △ charge MM-48, 1, 28-48
- charge MM-48, 4, 6-54.

approximate linear relations on logarithmic scales between the inherent viscosities in benzene and the various vistex solvents. There is, therefore, good evidence that valid viscosity - molecular weight relations may be obtained in the system toluene-isopropanol (80/20 by volume).

The correlation of intrinsic vistex viscosities and the intrinsic viscosities of the corresponding coagulated, dried polymer was then investigated. Experimental data are shown in Tables I and II and plotted in Fig. 3.

The least squares equation for these data is:—

$$\log [\nu]_T = 0.101 + 1.04 \log [\nu]_V. \quad (3)$$

Deviations of experimental points from the least squares line were considerably greater than those found for the coagulated, dried polymer, see Fig. 2. These are the result of additional variables introduced by coagulation and drying. Both negative and positive changes in $[\nu]_V$ of the same magnitude as the deviations shown in Fig. 3 have been found for latices under various conditions of aging even at low temperatures. Differences in $[\nu]_T$ or $[\nu]_V$ between Equation (2) and Equation (3) are of the order of only 0.05 dl. per

TABLE II

INTRINSIC VISTEX VISCOSITIES AND CORRESPONDING INTRINSIC VISCOSITIES OF COAGULATED, DRIED POLYMER IN TOLUENE. BUTADIENE-STYRENE COPOLYMER

No.	Parts DDM	Conversion, %	Intrinsic vistex	Intrinsic viscosity
<i>MM-48, 2, 4-49</i>				
1	0.42	20.0	—	1.24
2	"	31.6	1.14	1.36
3	"	41.4	1.19	1.53
4	"	—	—	—
5	"	69.8	1.75	2.20
6	"	75.0	2.02	2.66
7	"	80.7	2.11	2.89
8	"	82.6	2.18	2.81
9	"	87.0	1.79	2.44
10	"	93.5	1.22	—
<i>MM-48, 2, 18-50</i>				
1	0.40	66.0	1.26	2.25
2	0.48	66.6	1.54	1.93
3	0.56	67.2	1.39	1.62
4	0.64	67.6	1.05	1.38

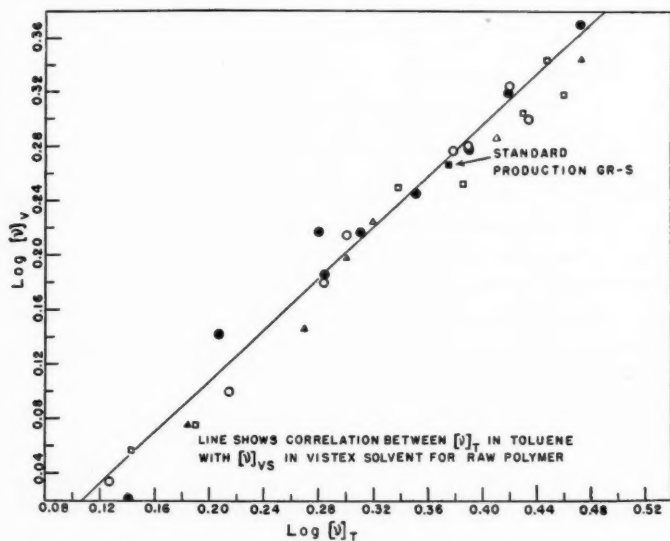


FIG. 3. Correlation between intrinsic viscosity and intrinsic vistex viscosity.

- charge *MM-48, 4, 6-54*
- charges *MM-48, 2, 19-51* and *MM-48, 2, 18-50*
- charge *MM-48, 2, 4-49*
- △ charge *MM-48, 1, 28-48*.

gm. Therefore, it is felt that Equation (2) can be used with confidence to calculate viscosity average molecular weights from intrinsic vistex viscosity data.

Vistex-Conversion Relations

The vistex method has been most widely used in the determination of vistex-conversion data. Typical intrinsic vistex viscosity - conversion and intrinsic viscosity - conversion curves are shown in Figs. 4 and 5 for varying conversions

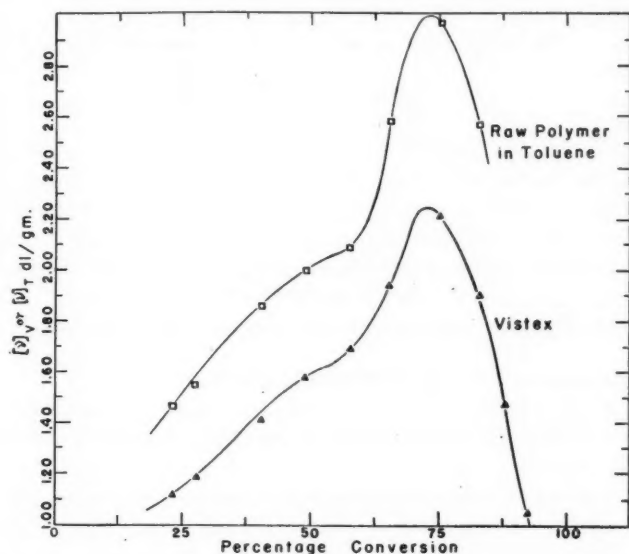


FIG. 4. Viscosity and vistex conversion curves for charge MM-48, 1, 28-48 (0.32 part DDM).

at two levels of modifier, 0.32 and 0.42 part of DDM. The maxima in the curves are very pronounced and appear to be shifted to higher conversions at higher levels of modifier, occurring at about 73% conversion for 0.32 DDM and about 80% conversion for 0.42 DDM.

The conversion level at which the maximum occurs is most commonly called the gel point. Owing to the general tendency to consider gel as detrimental to the quality of a rubber, the gel point is most frequently determined with the intention of restricting study to the system at conversions below this point or of delaying the onset of gel. Determination of the gel point is therefore, of considerable importance in polymerization research.

Although the gel point may be estimated fairly readily from vistex-conversion curves, a method of estimation of the position of a single determination relative to the gel point would be useful. One possibility of this lies in the slope of the inherent vistex viscosity vs. concentration lines.

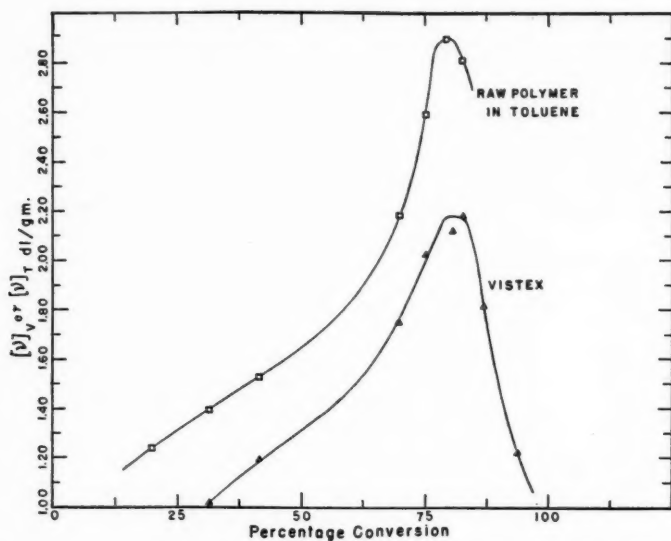


FIG. 5. Viscosity and vistex conversion curves for charge MM-48, 2, 4-49 (0.42 part DDM).

Slopes of the Inherent Viscosity vs. Concentration Lines

A typical series of lines relating the inherent vistex viscosity and concentration over a range of conversions is shown in Fig. 6. Within the limits of

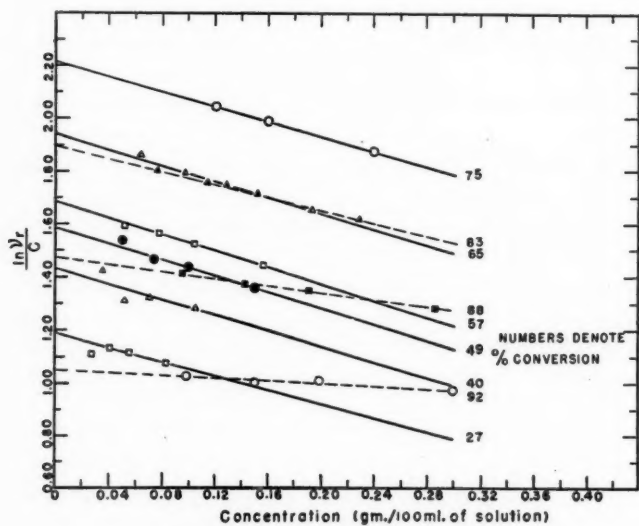


FIG. 6. Inherent vistex viscosity vs. concentration.

concentration used, the inherent vistex is related to the concentration of the polymer solution by the equation:—

$$\frac{\ln \nu_r}{c} = b - ac, \quad (4)$$

where b is the intrinsic vistex viscosity, $[\nu]_V$, while a is the slope of the line and reflects the interaction among solute molecules. Mead and Fuoss (16) found for a wide range of fractions of polyvinyl chloride that:

$$a = \beta[\nu]^2, \quad (5)$$

where β is a constant equal to 0.14. The resulting equation,

$$\frac{\ln \nu_r}{c} = [\nu] - \beta[\nu]^2 c, \quad (6)$$

has been derived by Huggins (13) from theory, using an approximation that is valid at zero concentration. However, Ewart (10) found that, when the slopes a of the inherent viscosity lines were plotted against intrinsic viscosities on a logarithmic scale, the resulting straight line had a slope considerably less than 2 for a butadiene-styrene copolymer. It was also found that the value of the exponent tended to approach 2.0 as the styrene content of the polymer increased. This suggests that the equation should be written:—

$$\frac{\ln \nu_r}{c} = [\nu] - \beta'[\nu]^n c, \quad (7)$$

where n is found experimentally for the material under investigation.

Baker (3) suggested that the slopes of the inherent viscosity lines might be used to detect gel and later (4) found that a sample of polybutadiene microgel showed strongly positive slopes and a low intrinsic viscosity. In Fig. 6 it is shown that the slopes of the lines representing polymers at high conversions, i.e., over the gel point, are distinctly less negative than those for samples of similar intrinsic viscosities at lower conversions. The same trend was found upon examination of the viscosity relations of coagulated, dried polymer in toluene. It appeared possible, therefore, that Equation (7) might be used to detect a significant change in viscosity characteristics at the gel point.

A test of Equation (7) is to plot the slope a of the inherent viscosity vs. concentration lines against intrinsic viscosity on a logarithmic scale. This plot is shown in Fig. 7 for the intrinsic vistex and for intrinsic viscosity data on the same series of polymers—varying conversion, 0.32 DDM. The slopes of the inherent vistex lines were corrected for water, using the data of Fig. 1. This correction is of the same order as that reported by Harris and Kolthoff (12) and was found to be valid in the range $[\nu]_T = 1.5$ –2.5. Values in the lower ranges of $[\nu]_V$ could not be corrected until more data on the variation of the correction for water at different values of $[\nu]_V$ are obtained.

The intrinsic viscosity data give a fairly good linear relation up to 65% conversion, with a slope n of 1.1 and $\beta' = 0.28$, i.e., the data up to 65% conversion are represented by

$$\frac{\ln \nu_r}{c} = [\nu]_T - 0.28[\nu]_T^{1.1}c. \quad (8)$$

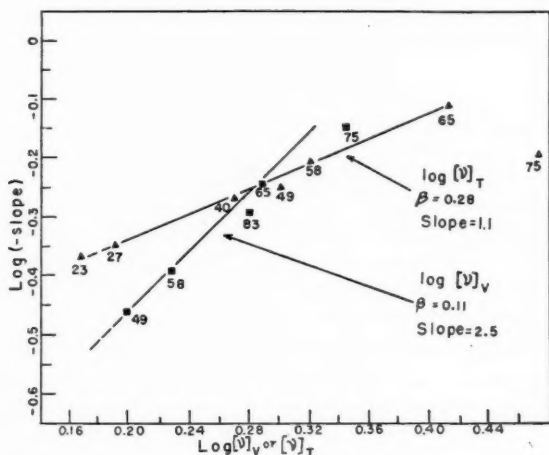


FIG. 7. Correlation between the intercept and slope of $\frac{\ln \nu_r}{c}$ vs. c for charge MM-48, 1, 28-48 (0.32 part DDM).

Above 65% conversion the values of slope a all lie below the values calculated from Equation (8) and this becomes very pronounced above 75% conversion. Values of β' calculated from Equation (8) change significantly from 0.28 at 65% conversion to 0.19 at 75% conversion. The change in β' correlates well with the onset of gelation in this case.

Intrinsic viscometry data shown also in Fig. 7 follow the same general trends and may be represented, up to 65% conversion, by the equation:—

$$\frac{\ln \nu_r}{c} = [\nu]_V - 0.11[\nu]_V^{2.5}c. \quad (9)$$

Data for the intrinsic viscosities of coagulated, dried polymer in the toluene-isopropanol (80/20) can also be represented by Equation (9). As might be expected, the relative change in calculated values of β' in the region of the gel point is much smaller in magnitude than that shown by polymer in toluene. However, the decrease in β' is still detectable at 75% conversion. Similar effects have been found in unpublished work on the viscosity relations of fractions of GR-S in toluene. The changes in the relations were tentatively ascribed to an increase in the branched structure of the molecule. The higher values of the exponent n in Equation (9) are apparently caused by the addition of nonsolvent. Mark *et al.* (11) have reported values of intrinsic viscosities,

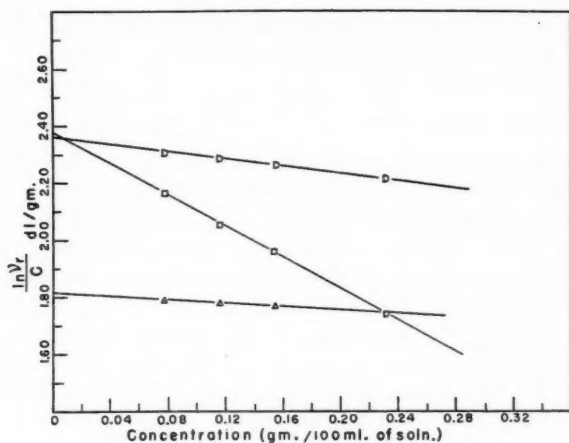


FIG. 8. $\frac{\ln v_r}{c}$ vs. c for coagulated, dried polymer.

- In toluene, diluting with toluene.
- △ In toluene-isopropanol (80/20), diluting with same.
- In toluene-isopropanol (80/20), diluting with toluene.

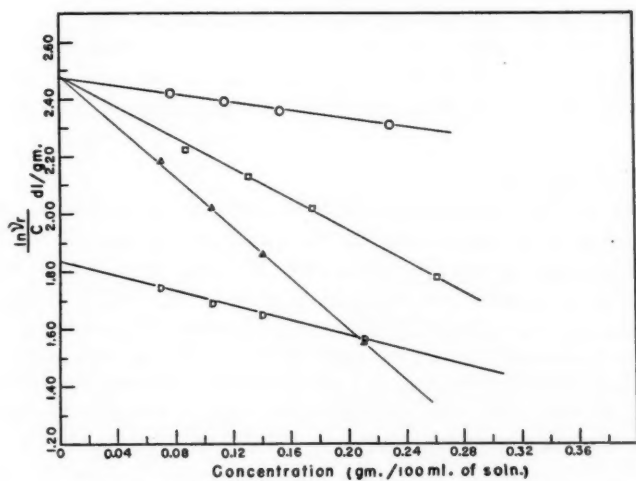


FIG. 9. $\frac{\ln v_r}{c}$ vs. c for polymer and latex solutions

- Polymer in toluene, diluting with toluene.
- Polymer in toluene-isopropanol (80/20), diluting with toluene.
- △ Latex in toluene-isopropanol, diluting with toluene.
- ◇ Latex in toluene-isopropanol, diluting with toluene-isopropanol.

slopes of the reduced specific viscosity vs. concentration lines, and values of k' calculated from the Huggins-Kraemer relation,

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \quad (10)$$

for fractions of polystyrene in toluene and methyl ethyl ketone (MEK). Values of k' for polymer in MEK were higher than those found with toluene and were not constant over the range of molecular weights when calculated from Equation (10). However, recalculation of the data has shown that the exponent is no longer equal to 2 for the MEK system. Substitution of the corrected value of the exponent in Equation (10) results in fairly constant values of k' . We are unable to compare the changes in exponent caused by addition of nonsolvent to that shown by our data because Mark's viscosity values for polymer in MEK were obtained at a higher temperature than those in the toluene system.

Other comparative data for whole polymers do not appear to be available in the literature. However, equations of this type have not been tested extensively for whole polymers, probably because it has been reported (18) that the values of slopes a of the inherent viscosity lines are affected by the heterogeneity of molecular weight distributions.

It is interesting, nevertheless, to note that the reduction in β' caused by the presence of 20% by volume of isopropanol, cf. Equations (8) and (9), is of the same order as the reduction of β caused by the addition of 20% by volume of butanol to a toluene solution of polystyrene reported by Alfrey *et al.* (2). The available data are not extensive enough to be used as a basis for correlation of β' and n with structural changes but they do suggest possibilities for further research.

Dilution with Pure Solvent

The data of Fig. 1 suggest that the vistex solution might be diluted with a pure solvent such as toluene and, by the use of the appropriate solvent flow times, the intrinsic viscosity in pure solvent obtained by extrapolation. This was found to be possible, as shown in Figs. 8 and 9. Excellent agreement between the intrinsic viscosities of coagulated, dried polymer in toluene and those of polymer and of latex in vistex solvent, diluting with pure toluene, were found at these viscosity levels. Although this method has a disadvantage in the greater slope for the inherent vistex line, it offers some interesting possibilities in polymerization research.

Acknowledgments

The authors wish to acknowledge the assistance given by Dr. H. L. Williams, Mr. M. Mitchell, and Mr. L. Fordham in the preparation of the latices and polymers, and for their helpful suggestions. We wish to express also our gratitude to Mr. E. A. May who carried out the viscosity measurements.

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NOTE ON THE THEORY OF ELECTROLYTIC DOUBLE LAYERS¹

BY A. J. DEKKER

Abstract

The mechanism suggested by Gurney for the formation of a double layer at the interface of a metal and a solution containing its ions is applied to a diffuse double layer. The diffuse part of the double layer is treated in a way that differs from Stern's method, leading to a more convenient formula for the potential ψ_δ of the diffuse part. Numerical values and a comparison with Stern's results are given.

1. Introduction

If a metal is dipped into an electrolytic solution, an electric double layer is generally formed. In the old theory of Helmholtz it was assumed that the metal acquired a surface charge, thus forming a molecular condenser with ions of opposite charge in the solution which are adsorbed on the metal. According to this theory, the whole potential difference between metal and bulk solution is confined to this nondiffuse double layer, and the capacity is given by $C_0 = \epsilon'/4\pi\delta$, where δ is of the order of an ionic radius and ϵ' is the dielectric constant of the layer. From experimental results it is known that C_0 is of the order of magnitude of $20\mu\text{f.}$ for an electrode of 1 cm.^2 It is difficult to assign a physical meaning to ϵ' and so it is perhaps better to speak about an effective distance $\delta_{\text{eff.}} = \delta/\epsilon'$ of the plates, referred to vacuum.

The idea of a diffuse double layer was first conceived by Gouy and Chapman to take into account the thermal motion of the ions and the diffusion resulting from it. Using Poisson's equation and a Boltzmann distribution, a relation between the surface charge σ_0 on the metal and the total potential drop ψ_0 is obtained. Owing to the fact that they consider the ions as pointlike particles, the calculated capacities are too high by a factor of about 10.

To account for the finite size of the ions, Stern (2) combined the foregoing ideas. According to him the total potential drop takes place in two stages: the first stage lies between $0 < x < \delta$ (see Fig. 1, c) and is commonly called "Stern layer", the second is a diffuse part in the region $x > \delta$ (Gouy layer). The value of δ is again of the order of an ionic radius. We so arrive at three representations (see Fig. 1), of which for the case of electrolytes Stern's is doubtless the best. There is, however, one point that we think is not completely satisfactory; Stern divides the Gouy layer into two parts, one being a surface charge at $x = \delta$ and the other part being really diffuse for $x > \delta$; the reason for this is not quite clear and we will see in Section 3 that a more convenient expression is obtained by considering the diffuse part as a whole.

As far as the mechanism of formation of a double layer is concerned, a theory has been put forward by Gurney (1) for the case of a metal dipped into a

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Contribution from the Department of Physics, The University of British Columbia, Vancouver, Canada.

solution containing its own ions. This mechanism is based on the assumption that the exchange of positive ions between metal and solution takes place by quantum-mechanical tunnel effect. If we apply this mechanism to a diffuse double layer, we obtain nearly the same result given by Gurney; this will be shown in Section 2.

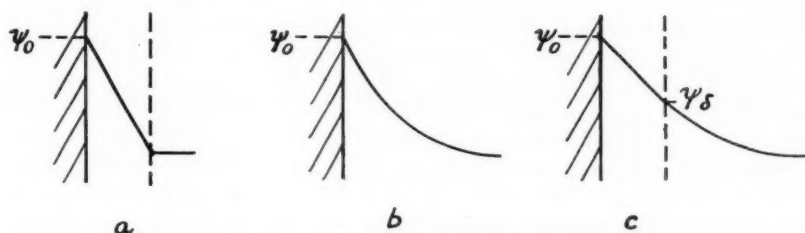


FIG. 1. Three representations of an electrolytic double layer

- a. Helmholtz
- b. Gouy-Chapman
- c. Stern

2. Calculation of ψ_0

Following Gurney's suggestion we will denote the energy of an ion on the metal surface in the ground state by Y_0 ; the energy of an ion at infinity will be taken as zero and the energies below zero will be counted as positive throughout this note. For a metal in vacuum, Y_0 is approximately equal to the sum of the sublimation and ionization energy minus the work function of the metal. The ground state of a hydrated metal ion in the solution will be denoted by W_0 ; for the moment we will assume that the metal ions are hydrated by one water molecule only. A complete description of the exchange mechanism between metal and solution is given by Gurney (1). The exchange process in each individual case will not always take place at the same distance between an occupied level on the metal and a vacant level in the solution, but we will assume an average distance δ . We will also assume that the negative ions in the solution do not approach the metal any closer than δ and hence in this model there is no space charge in the region $0 < x < \delta$, in accordance with Stern's idea.

Suppose now that in the equilibrium state, for which the number of ions going into solution is equal to the number deposited, the total potential drop between metal and bulk solution is ψ_0 , where ψ_0 has the sign corresponding to the metal. Referred to the energy levels in the bulk solution, the ground state of an ion on the metal surface will then have changed into $(Y_0 - e\psi_0)$ and hence the fraction of surface ions with energies between E and $E + dE$ is for a Boltzmann distribution given by:*

$$\exp. (E - Y_0 + e\psi_0)/kT \cdot dE/kT.$$

* We consider monovalent ions throughout.

In the same way the fraction of hydrated ions at $x = \delta$ having energies in the same range will be equal to

$$\exp. (E - W_0 + e\psi_\delta)/kT \cdot dE/kT.$$

If N_w is the number of water molecules at $x = \delta$ per unit area opposite the metal and N_δ^+ the corresponding quantity for the positive ions, we obtain the equilibrium condition

$$N_w p_s(E) \exp. (E - Y_0 + e\psi_0)/kT \cdot dE/kT \\ = N_\delta^+ p_d(E) \exp. (E - W_0 + e\psi_\delta)/kT \cdot dE/kT \quad (1)$$

Here $p_s(E)$ is the probability that a surface ion with energy E will jump to a corresponding level in the solution per unit time; $p_d(E)$ is the corresponding term for deposition. The ratio $p_d/p_s = \beta$ will actually be a function of E , but the process of exchange will take place in a very narrow band of energies and will be treated as a constant; β is probably of the order of 1.*

We will now define the concentration of the bulk solution as

$$c_\infty = \text{moles of salt/moles of water.}$$

Writing $c_\delta^+ = N_\delta^+/N_w = n_\delta^+/n_{w\delta}$, where n refers to numbers per unit volume, it can easily be seen that if we assume the number of water molecules per unit volume to be approximately the same for $x = \delta$ as for $x = \infty$,

$$c_\delta^+ \simeq c_\infty e^{-e\psi_\delta/kT}. \quad (2)$$

Expressions (1) and (2) lead to the result

$$e\psi_0 \simeq Y_0 - W_0 + kT \log \beta c_\infty. \quad (3)$$

This result is the same as that obtained by Gurney, except for the slight approximation just mentioned.

To correct for the fact that a positive ion probably polarizes more than one water molecule, we could introduce a correction similar to that introduced by Gurney. Suppose each positive ion is on the average hydrated by p water molecules. In this case N_w in Equation (1) should be replaced by $(N_w - pN_\delta^+)$ and the factor βc_∞ in Equation (3) by $\beta c_\infty / (1 - pc_\delta^+) \simeq \beta c_\infty e^{pc_\delta^+}$. Defining $\log f^+ = pc_\delta^+$ we would find instead of Equation (3)

$$e\psi_0 = Y_0 - W_0 + kT \log \beta f^+ c_\infty. \quad (3a)$$

For $c_\delta^+ \rightarrow 0$, f^+ approaches 1. This case is different from that of a non-diffuse double layer inasmuch as in the latter case the "activity coefficient" would be defined by $\log f = pc_\infty$ (see Gurney). The relation between f^+ and f is apparently

$$\log f^+ = e^{-e\psi_\delta/kT} \log f.$$

For $e\psi_\delta \gg kT$ the difference between f^+ and f becomes appreciable, but as far as the value of ψ_0 is concerned, the term $kT \log \beta f^+ c_\infty$ is generally small compared with $(Y_0 - W_0)$ and the correction for the value of ψ_0 in this case

* A more detailed statistical treatment of this equilibrium between a metal and an ionic atmosphere is given by Fowler in an appendix to Gurney's article.

will be small. Considering some numerical values, let us suppose $p = 3$. Then in a 1 normal solution $c_\infty \approx 1/55$ and $\log f = 0.055$; the uncorrected term in Equation (3) would give

$$kT \log \beta c_\infty \approx -4kT.$$

For positive values of ψ_δ the factor $e^{-e\psi_\delta/kT}$ is always smaller than 1 and this will lead to $\log f^+ < \log f$ and hence a correction of about 1% or less. If ψ_δ is negative, as could conceivably happen in certain cases, the correction becomes larger and will depend on the ratio $e\psi_\delta/kT$. Taking $\psi_\delta = 0.1$ volt, one would get $kT \log \beta c_\infty \approx -4kT$ instead of

$$kT \log \beta f^+ c_\infty \approx -kT,$$

or in other words a total correction of $3kT$ for $e\psi_0$. However, in concentrated solutions, the value of ψ_δ for a given value of ψ_0 is always smaller than in diluted solutions, and this effect depresses this correction further.

3. Calculation of ψ_δ

In the Stern layer, between $x = 0$ and $x = \delta$ we have

$$\frac{d^2\psi}{dx^2} = 0; \text{ hence } \psi_0 - \psi_\delta = \frac{4\pi\delta}{\epsilon'} \sigma_0,$$

where σ_0 is the surface charge-density on the metal. Here, as already remarked in the introduction, it is perhaps better to introduce an effective plate distance $\delta_{eff} = \delta/\epsilon'$. For the region $x > \delta$ Poisson's equation gives

$$\frac{d^2\psi}{dx^2} = -\frac{4\pi}{\epsilon} \rho(x);$$

in this whole region we will assume a constant value of ϵ .

Writing n_∞ for the number of ions of each type per unit volume in the bulk solution, this equation becomes

$$\frac{d^2\psi}{dx^2} = \frac{8\pi n_\infty e}{\epsilon} \sinh \frac{e\psi}{kT}.$$

Multiplying both sides by $\frac{d\psi}{dx} dx$, this leads on integration to

$$\frac{d\psi}{dx} = -\left(\frac{32\pi n_\infty kT}{\epsilon}\right)^{1/2} \sinh \frac{e\psi}{2kT}. \quad (4)$$

To find an expression for ψ_δ we can make use of the relation

$$\int_\delta^\infty \rho(x) dx = -\sigma_0$$

or

$$2en_\infty \int_\delta^\infty \left(\sinh \frac{e\psi}{kT}\right) dx = \sigma_0 \quad (5)$$

Introducing in Equation (5) $d\psi$ instead of dx by means of Equation (4) and integrating leads to the following expression

$$\sigma_0 = \left(\frac{n_{\infty} k T \epsilon}{2\pi} \right)^{\frac{1}{2}} \cdot 2 \sinh \frac{e\psi_{\delta}}{2kT}. \quad (6)$$

We can now eliminate σ_0 , giving

$$\psi_0 - \psi_{\delta} = 8\pi\delta_{eff} \left(\frac{n_{\infty} k T \epsilon}{2\pi} \right)^{\frac{1}{2}} \sinh \frac{e\psi_{\delta}}{2kT}. \quad (7)$$

This expression is, as a matter of fact, completely independent of the way in which ψ_0 is determined. In the case of a metal in a solution of its ions, ψ_0 is determined by Equation (3); in other cases ψ_0 can be determined by an electronic equilibrium in the same way as for instance in the case of a metal in contact with an insulator or semiconductor.

Equation (7) enables us to calculate ψ_{δ} for a given value of ψ_0 as a function of the concentration of the bulk solution. Instead of one term at the right-hand side of Equation (7), Stern obtains two terms because he divides the diffuse layer into two parts; we think, however, that it is more consistent not to do this.

4. Numerical Examples

Starting from Equation (7) we will give some numerical values of ψ_0 calculated for a given ψ_{δ} and concentration. To be able to compare the results with those of Stern, we will take the same value, $C_0 = 1/4\pi\delta_{eff} = 2.6 \times 10^7$ cm., that he used. This value is taken from the work of Krueger and Krumreich* on the electrocapillary curve of the system mercury-1 normal potassium nitrate: from their measurements it can be concluded that the capacity of this system is independent of ψ_0 , or, in other words, that the diffuse part of the double layer is negligible compared with the Stern layer. Assuming $\epsilon = 81$ we find on inserting the various constants into Equation (7) for the potential difference expressed in volts that

$$\psi_0 - \psi_{\delta} = 0.42 \sinh \frac{e\psi_{\delta}}{2kT} \quad \text{for a 1 normal solution}$$

$$\psi_0 - \psi_{\delta} = 0.042 \sinh \frac{e\psi_{\delta}}{2kT} \quad \text{a 0.01 normal solution.}$$

Some results are given in Table I; Stern's corresponding values are included (S). As far as the capacity $C = \sigma_0/\psi_0$ of the diffuse layer is concerned, we see that because $\psi_0 - \psi_{\delta} = 4\pi\delta_{eff}\sigma_0$ and $C_0 = 1/4\pi\delta_{eff}$ (being the capacity of a flat layer), $C = C_0(1 - \psi_{\delta}/\psi_0)$. Hence, C is 100 ψ_{δ}/ψ_0 % less than C_0 . These percentages are also included in the table.

The conclusions that can be drawn from this table are qualitatively the same as those obtained from Stern's more complicated formula. At higher concentrations the double layer becomes flatter and in a 1 normal solution the capacity is only slightly dependent on ψ_0 ; in dilute solutions, however,

* See Stern (2).

TABLE I
NUMERICAL RESULTS (SEE TEXT)

ψ_δ	1 normal solution				0.01 normal solution			
	ψ_0		100 ψ_δ/ψ_0		ψ_0		100 ψ_δ/ψ_0	
0.025	0.24	—	10	—	0.047	—	53	—
0.05	0.54	S 1.06	9.3	S 4.5	0.10	—	50	—
0.10	2.0	—	5.0	—	0.25	S .302	40	S 33
0.15	—	—	—	—	0.57	S .914	26	S 16.4
0.20	—	—	—	—	1.33	—	15	—

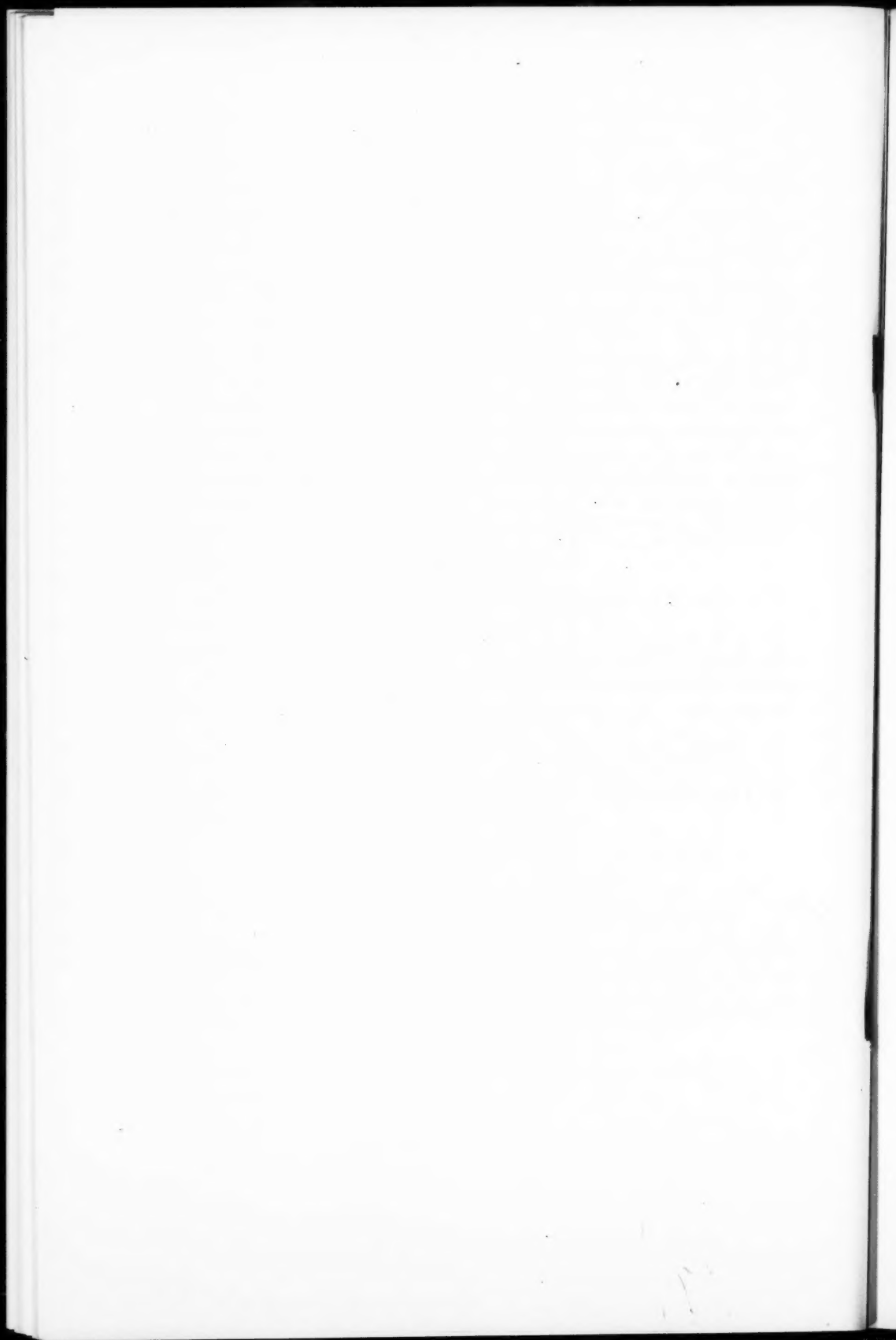
the capacity is strongly dependent on ψ_0 , and especially for small values of ψ_0 the difference between C and C_0 is appreciable. In general, Stern's values of ψ_δ corresponding to the same ψ_0 are smaller than those obtained from Equation (7). We do not think it possible at the moment, however, to decide which agree best with the experiments.

Acknowledgment

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